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Edited at The University of Texas Medical Branch, Galveston

OPPORTUNITIES FOR DETERMINING SEX IN HUMAN TISSUES

T. C. Hsu, C. A. Hooks, and C. M. Pomerat*

In urological practice the complex morphological anomalies of the external genitalia may not be resolved by the examination of gonadal biopsies. Hermaphrodism in man, though rare, constitutes an important challenge to surgical decision. Severinghaus (1942) was probably the first investigator who studied the meiotic chromosomes in a human pseudohermaphrodite. His case showed the X–Y pair in the spermatocytes. Recently Barr and his associates (Barr, *et al.*, 1950; Moore, *et al.*, 1953) have suggested that sex differences in the distribution of the heterochromatins of the interphase nucleus of somatic cells might be useful in determining the sex of such individuals.

While gonadal biopsy is not always available to provide clear-cut meiotic division to show the sex-pair, complementary evidence of the "basic sex" in human hermaphrodites can be obtained by examining the chromosomes of somatic cells. It is well known that the chromosomes of cells dividing in tissue culture are in a relatively flat plane and therefore can be studied without the use of sectioning technique. A further advantage to this approach was suggested (Hsu and Pomerat, 1953) with the use of prefixation treatment of cells in culture with a hypotonic solution which results in a remarkable spreading of chromosomes. In practice, the method consists of treating cultures showing large numbers of mitoses with a mixture of 15–20 parts of Gey's balanced salt solution with 85–80 parts of the same formula but from which NaCl has been omitted. Following 20 minutes in the presence of the hypotonic solution at body temperature, the cultures are generally fixed with methanol and stained with a combination of May-Grünwald and Giemsa (Jacobson and Webb, 1952) or by fixation in Helly Zenkers and stained overnight in diluted Delafield haematoxylin.

* From the Tissue Culture Laboratory and the Department of Urology, Medical Branch, The University of Texas, Galveston. Received for publication November 2, 1953.

It is assumed that any somatic tissue may prove valuable for determination of sex but due to practical considerations explants of full thickness skin or bone marrow obtained under general anesthesia were employed. While improvements in the technique of bone marrow culture are needed, the skin, especially the spindle cells from the dermis, provide a reasonably useful test object. It has been considered wise to sample tissues from both the right and left sides of the body.

Thus far, cytological preparations have been obtained from eight individuals. In fortunate instances the Y-chromosome has been clearly identified in an isolated position (compare Figs. I and II). Such cells give relatively sure proof for the identifi-

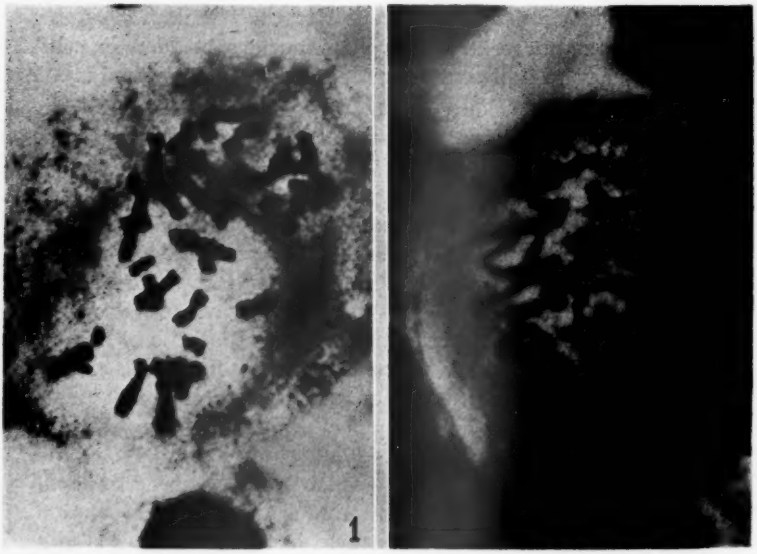

FIG. I. Chromosomes of a normal male. Note the short Y-chromosome at the lower left corner.
FIG. II. Chromosomes of a male-type intersex. Note the Y-chromosome at one-o'clock position.

cation of the male configuration in terms of what is known of the size of this chromosome. Our experience has suggested that the Y-chromosome is probably shorter than that given in a

provisional diagram by Hsu (1952). Matching of the chromosomes involves considerable labor and can be done only with perfectly spread preparations. However, in female cases where no Y-chromosomes are present matching work should be done for at least a few cells.

While the work this far is still in its preliminary stages, it appears to offer an additional guide to the urologist who approaches the problem of extirpating one or the other of the rudimentary portions of an abnormal sexual apparatus and providing encouragement of the development of its opposite by plastic repair and hormonal therapy. It is recognized that such procedures should be instituted as soon as possible so that the program of social psychological adjustment of the individual can be established from early infancy. The combination of a tissue culture laboratory and an adequately trained personnel for the study of nuclear cytology is not sufficiently widespread to make the method commonly available but perhaps a few well-established centers could meet the special needs which arise in this somewhat restricted field.

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CHROMOSOME FEATURES IN THE REGENERATING RAT LIVER FOLLOWING PARTIAL EXTIRPATION

SAJIRO MAKINO AND TATSUYA TANAKA*

For several years the authors have studied the cytology of ascites tumors of rats (Makino, 1952; Makino and Kanô, 1953; Tanaka and Kanô, 1951) with particular concern with the mechanism of chromosomal changes occurring in ordinary tissue cells which by the application of chemicals are induced to form tumors. Need for a more complete knowledge of the somatic chromosomes of rats has led the authors to undertake this study. This short paper is to report briefly the chromosome phenomena observed in the regenerating liver of adult rats following partial extirpation.

The full-grown rats, 100 to 150 gm. in body weight, were starved for about 24 hours before operation. The animals were then anaesthetized and the median and left lateral lobes of the liver were removed. This represents about 40 per cent of the total liver mass. The rats were sacrificed at various intervals after operation, such as 20, 24, 48, and 96 hours. Most of the cytological preparations used were prepared by the squash method devised by Tanaka (1951). The procedure is as follows: tissues are cut into small pieces and fixed with acetic alcohol (1:3) for 20 to 60 minutes according to the size of the tissue. The tissues are then immersed in 10 per cent acetic acid for 5 minutes, and transferred to and left in 50 per cent acetic acid for 15 to 30 minutes. The tissues thus treated are stained with acetic orcein or acetic gentian violet (1 gm. of gentian violet in 100 cc. of 45 per cent acetic acid) for 30 to 50 minutes. Then the tissues are squashed on a slide by applying an even pressure to the cover slip.

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* From the Zoological Institute, Hokkaido University, Sapporo, Japan. Experiments described in this paper were done in the Zoological Institute of Hokkaido University, and the preparation of the manuscript was accomplished by the senior author while he was a guest investigator in the Department of Zoology, The University of Texas, Austin. The senior author wishes to thank Dr. T. S. Painter, Dr. C. P. Oliver, and Dr. M. J. D. White for extending the generous hospitality of the laboratory. Received for publication July 13, 1953.

Results and Conclusions

Mitotic division of the hepatic cells is generally rare in the normal liver of the adult rat. Partial removal of the liver is followed by rapid mitotic multiplication of cells in the remaining part. Especially during the period between the first and second day after operation, the mitotic figures are plentiful in the tissue undergoing restoration.

The rate of mitosis was firstly studied in the regenerating liver. One thousand hepatic cells were observed at each of the indicated intervals through the process of restoration, and the frequency of mitotic cells per interval of time was calculated in percentage on the basis of the above data. As shown in table 1, the results indicate that the proportion of mitotic cells showed a gradual increase from the early period (about 5 to 20 hours after operation) continuing towards the middle period (about 42 to 50 hours after operation) of the liver restoration. The frequency curve of the mitotic rate forms a peak at the middle period and then shows a decline towards the latter period of the restoration (about 72 to 90 hours after operation).

TABLE 1

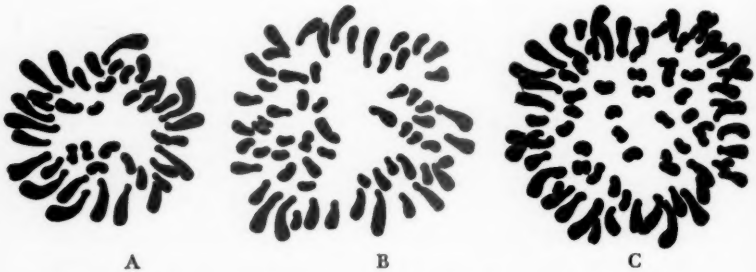
Frequency of Mitotic Cells in the Course of Liver Restoration after Partial Extirpation

Hours after operation	5	10	20	24	30	36	42	48	60	72	96
Per cent of dividing cells	0.3	1.3	2.4	3.9	3.6	3.9	4.5	4.6	3.0	2.2	0.9

Per cent of dividing cells was calculated on the basis of 1,000 cells in each period.

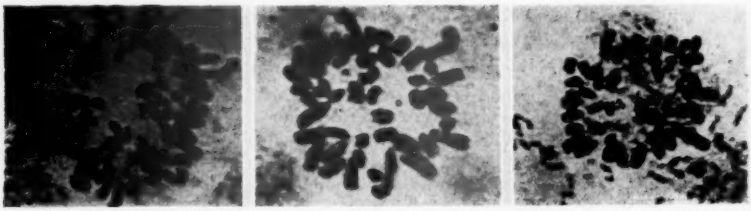
Based on the same preparations as were used for the study of the mitotic rate, the number of chromosomes in the regenerating liver cells in the process of restoration was also investigated (Fig. I, A-C; Fig. II, A-C). The results indicated that the number of chromosomes widely varied between two extremes, 36 for the smallest and 89 for the largest. It is noticeable, however, that the cells showing the basic chromosome number, 42, were highest in frequency. In a total of 1165 cells examined, 340 cells showed the basic number. The cells with any other chromosome number

were few in occurrence. The relative frequency of cells having 42 chromosomes tended to increase gradually with the passage of time after operation. They represent 34.8 per cent of the mitotic cells in the material 24 hours after operation, 29.8 per cent in the 48-hour material, 41.5 per cent in the 72-hour material and 44.8 per cent in the 96-hour material.



A B C

FIG. I, A–C. Chromosomes from regenerating liver cells. 42, 64, and 84 chromosomes in each. X 3000.



A B C

FIG. II, A–C. Photomicrographs of the chromosomes from regenerating liver cells. A and B; cells of diploid range. C, cell of tetraploid range. X 2000.

In spite of a wide variation in chromosome number, the cells tend to fall into three definite ranges: namely, with chromosome numbers ranging from 36 to 46, 59 to 68, and 78 to 89. The cells having 36–46 chromosomes were most frequent in occurrence, with those of 78–89 chromosomes coming next. The three groups here obtained interestingly correspond to the diploid range ($\pm$ 42), triploid range ($\pm$ 63) and tetraploid range ($\pm$ 84). It is remarkable to see that the cells in the diploid range were always frequent in occurrence in the regenerating liver, and further that the proportion of those cells increased from approximately one-

half of the cells in the early period of the liver restoration to 80 per cent in the latter period (Table 2). From the above evidence

TABLE 2

Frequency of the Cells of the Diploid, Triploid and Tetraploid Ranges

Hours after Operation	Number of cells Diploid Range (36–46 chroms.)	Triploid Range (59–68 chroms.)	Tetraploid Range (78–89 chroms.)
20	43	7	13
24	51	19	31
30	105	59	47
36	103	39	48
42	97	18	41
48	135	25	43
72	86	10	21
96	58	3	11
Total	668	180	255

it can be said that the cells of the diploid range play a significant role with cooperation of the cells of the tetraploid and triploid ranges, in the regeneration of the liver after the partial extirpation.

In the hepatic cells, a binucleate condition is not uncommon. The frequency of binucleate cells observed in the normal liver is given as about 27 per cent for young rats, 15 per cent for grown rats, and 17 per cent for old rats. In the animals under study binucleate cells were found to be more frequent in the normal liver than in the restoring liver. Following the partial removal of the liver the frequency of the binucleate cells decreased abruptly. Extreme decrease occurred during the period from 48 to 72 hours after operation. By this time the most active multiplication of regeneration cells has taken place. Near the latter period of liver restoration, the binucleate cells showed a gradual increase in frequency (Table 3). This seems to imply that the binucleate

TABLE 3

Percentage of Binucleate Cells in the Regenerating Rat Liver after Partial Removal

Hours after Operation	0	20	24	30	36	48	60	72	120
Per cent of binucleate cells	27	14	9.2	10.6	7.8	2.4	2.4	2.1	6.9

cells contribute little to restoration of liver after the partial removal.

Biesele, Poyner, and Painter (1942) described in the embryonic mouse liver a method of doubling chromosome numbers and size of nuclei without breakdown of the nuclear wall which they believe to be endomitosis. The authors have never observed evidence of endomitosis in restoring rat livers. Tanaka (1951) also failed to find endomitotic cells in various tissues of foetal rats.

Summary. The results of the present investigation indicate that, although there are a wide variation of the chromosome numbers in the liver cells of adult rats during restoration after partial extirpation, the most frequent chromosome numbers fall into the following three ranges: the diploid range (36–46 chromosomes), the triploid range (59–68 chromosomes), and the tetraploid range (78–89 chromosomes). Since the cells of the diploid range, particularly those containing 42 diploid chromosomes, exist in the highest frequency through the course of liver restoration, they probably contribute primarily to the restoration of the liver after extirpation.

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A CLINICAL COMMENTARY ON THE USE OF SUCCINYLCHOLINE DICHLORIDE IN ELECTRO-SHOCK THERAPY

NEVILLE MURRAY*

Succinylcholine dichloride has been investigated by several workers in physiodynamic therapy since the drug was first employed in modified electro-shock therapy. Holt and Wilson, as well as others, have described the use of this drug in the poor risk patient, with detailed observations on cardiographic and other vital phenomena. Alexander has described its use in electro-stimulatory procedures. All of these reports, including an earlier one by the writer, have been most enthusiastic in praise of this agent.

Kalinowski has reported (personal communication) of two fatal cases attributed to the use of this preparation which occurred in Europe, and I have had experience of one cardiac death occurring in a seventy-seven year old poor risk patient, which was associated with, but not attributed to, the use of this drug.

It is my purpose here to document some further experience with "Anectine" (a trade-name for succinylcholine dichloride) in a total of approximately 15,000 treatments, given to some 1,500 patients of varying age group and sex, treated at the Medical Branch of The University of Texas.

All of these patients were under the care of varied staff and resident psychiatrists, and consisted of both private and charity patients, all of whom were treated on a voluntary basis. The routine treatment technique used consisted of the administration of electro-shock therapy simultaneously with the intravenous injection of succinylcholine dichloride, *without* the use use of barbiturate anesthesia.

Our objections to the use of barbiturate are several. Firstly, the dangers of treatment are markedly increased by the addition of a basal anesthetic and depressant agent, combined with

* From the Department of Neuro-Psychiatry, The University of Texas Medical Branch, Galveston. Presented at the Mid-Continental Psychiatric Association, Kansas City, Missouri, September, 1953.

a peripheral respiratory depressant in the form of a curare-like substance. Secondly, we have found that vomiting occurs much more frequently with intravenous Pentothal (thiopentone). Thirdly, there is an antipathetic effect between the two substances, succinylcholine and thiopentone. The dose of succinylcholine has to be markedly increased, in order to bring about the equivalent relaxation when the two are used together. This fact probably accounts for the larger average doses used by other workers. We have never been required to administer more than twenty milligrams to one patient, and have always achieved adequate results. Fourthly, it is impossible to observe the degree of relaxation by asking the patient to perform a voluntary act, gripping the hands, or snapping the fingers when consciousness has been obliterated by means of basal anesthesia. And, lastly, the recovery rate after Pentothal anesthesia is markedly prolonged, and the patient is intoxicated with barbiturate for some hours after treatment; whereas, in the non-pentothal patient, the daily recreation or subsequent therapeutic interview can be carried out with minimal treatment after-effects. It is felt that by using our technique, the subjective discomforts to the patient can be overcome without basal anesthesia.

Technique of Combined Insulin and Electro-Shock Therapy. In the case of combined insulin and electro-shock therapy, the procedure adopted was as follows: Patients, after the prescribed period of coma, were interrupted by intravenous administration of 50 cc.'s of fifty percent glucose. The practice of tube feeding patients before electro-shock therapy is not recommended, since the possibility of expulsion and aspiration of abdominal contents forms an additional danger. As soon as the patient showed evidence of purposive behavior after the injection of the glucose, the succinylcholine was given, and the patient was then observed for evidence of peripheral paresis. The characteristic fasciculations seen in the routine treatment without insulin are not always seen, and the more definite signs that adequate paralysis exists is evidenced by a decrease in the amplitude of thoracic respiratory movement, and the observation of contractions of the nares and other accessory muscles of respiration. The time taken for paralysis to be established is of a somewhat longer duration in patients receiving insulin, and is usually of the order of thirty to forty seconds after the end of the injection of succinylcholine. Thereafter, the electrical stimulus is administered in the usual way. It is not our practice in our clinic to give electro-shock therapy in the deepest stage of coma, as has sometimes been recommended. Under these circumstances, the administration of a respiratory

depressant drug where a considerable degree of cerebral anoxemia may already co-exist, is, in my opinion, a highly dangerous practice.

Further Precautions. In routine treatment, when succinylcholine has been injected paravenously by mistake, the effects of the drug are minimal, and insufficient for therapeutic purposes. It is our custom to repeat the injection of the same dose of the drug if the patient has not shown adequate responses after forty to forty-five seconds from injection time. Since the drug is rapidly destroyed in the tissues, there are no cumulative effects, and failure to repeat the injection resulted in a compression fracture of the lumbar vertebra in one patient. This was described in our previous report. A warning, too, against the practice of pressing forcibly on the chest wall in order to promote respiration. Where apnoea does occur, it is necessary to inflate the lungs directly by means of oxygen under positive pressure. Pressing on the chest wall, manually, might well bring about bruising and trauma to the soft tissues no longer protected by the tonus of thoracic musculature.

The only other case of fracture which we have encountered in our entire series, occurred in a poor risk patient who suffered from chronic rheumatoid spondylitis of twenty years' standing. He was a thirty-eight year old man, and had a complete spinal-sacral-iliac fusion, resulting in a "poker back" deformity. On the fifth treatment, the patient, who had been treated successfully in a "sitting-up" position, developed difficulty in maintaining an airway during recovery. This was associated with his neck deformity, and he required emergency rescusitation. On the sixth treatment we unwisely decided to reduce the dose of succinylcholine from the previous fifteen milligrams to ten milligrams. As a result, the patient suffered a fracture of the femoral neck, and the acetabulum, during convulsion.

Lactic Acid Studies. One of the most interesting studies which has been carried out since we have begun to use anectine electro-shock, is an investigation carried out by Drs. Harris, Nowinski, and Mitis (personal communication), on changes in the lactic acid level of the blood and spinal fluid following electro-shock therapy, with and without "Anectine." These changes showed that the normal level of lactic acid in blood is doubled or trebled after electro-shock in those patients receiving succinylcholine premedication. In the same patients who received electro-shock without the benefit of a relaxant drug. the increase in lactic acid was eight to ten times the normal level. No changes were found in spinal fluid levels.

Since these changes in lactic acid level are not related to therapeutic results of electro-shock treatment, these important observations are somewhat contrary to the findings of Lowenbach and Greenhill, who claimed improvement in fifty percent

of depressed cases by administering large doses of lactic acid by mouth. The lower lactic acid blood levels in succinylcholine electro-shock treatment might well explain the striking absence of after-treatment fatigue in our patients.

Clinical Impressions. It is that absence of post-treatment complaints which has impressed my colleagues and myself with the efficacy of this method of electro-shock treatment. The fact that we have administered this technique over 15,000 times with minimal trauma is in itself a convincing argument, but to the individual patient, it is much more pertinent that he awakens from his treatment with little or no muscular discomfort, and without broken teeth or a bitten tongue. To the nursing staff it has brought about the discontinuation of the unpleasant practice of holding down the jactating patient, and the welcome absence of the eerie "epileptic cry." To the resident it has meant the use of a quick and an easily administered treatment, without the use of glissando apparatus or other complicated electrical machinery. To the staff psychiatrist it has allowed the prescribing of electro-shock treatment in poor risk patients, who previously would have been denied such benefits and, in the routine case, he is enabled to practice without the threat of legal malpractice suit.

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EFFECT OF TETRAETHYL THIURAM DISULFIDE (ANTABUS) ON THE ALCOHOL METABOLISM *IN VIVO**

WIKTOR W. NOWINSKI AND PAUL L. EWING†

Hald and Jacobson demonstrated in 1948 that individuals sensitized with tetraethylthiuram disulfide (Antabus), show a very high accumulation of acetaldehyde in the blood within 30 minutes after ingestion of small amounts of ethyl alcohol. In order to explain this phenomenon, Westerfeld *et al.*, Richert *et al.*, and Richert and Westerfeld investigated the activity *in vitro* of xanthine oxidase in the presence of Antabus and found a marked inhibition of this enzyme. They concluded that also in the living organism, accumulation of acetaldehyde after ingestion of Antabus must be due to the inhibition of xanthine oxidase. As the Schardinger enzyme from milk was not affected by tetraethylthiuram disulfide, the authors concluded that although these enzymes are similar in structure, the sensitivity to Antabus reveals a difference due to a not yet identified constituent to which the liver xanthine oxidase is attached.

Contrary to the findings of Westerfield *et al.* Kjelgaard, using highly purified preparations of aldehyde oxidases from rabbit liver, showed that *in vitro*, Antabus primarily affects these enzymes. He also confirmed that the Schardinger enzyme is not inhibited, but interpreted this finding as being evidence against the possible involvment of xanthine oxidase in this inhibition. This point of view was supported by the results of Kalckar, Kjelgaard, and Klenow, who obtained inhibition of both the enzyme from milk and from the liver, by using 2-amino-4-hydroxy-6-formylpteridyl aldehyde, a photo-fission product obtained by Lowry, Bessey, and Crawford, thus demonstrating that the same inhibitor acts in both cases.

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* From the Tissue Culture Laboratory and the Departments of Neuro-Psychiatry and Pharmacology, The University of Texas Medical Branch, Galveston. Aided by a contract from the Office of Naval Research, N6onr-266 T.O.V., administered by Dr. C. M. Pomerat.

† Present address: Dept. of Pharmacology, University of Arkansas School of Medicine, Little Rock, Arkansas.

In face of this controversy, it seemed of importance to investigate *in vivo* whether or not Antabus is a specific inhibitor of xanthine oxidase, particularly because the experiments of Kjelgaard as well as those of Richard *et al.*, were performed *in vitro*. Pigeons were chosen as experimental animals, because Morgan showed in 1926 that their livers do not contain xanthine oxidase, a fact also confirmed recently by Richert and Westerfeld. The latter authors showed furthermore that pigeon tissues are in general very much depleted of this enzyme.

Methods. Healthy pigeons, weighing 260–600 grams were chosen for experiments and kept on standard diet, consisting of Rabbit Chow, Purina Rat Chow, white bread and milk. The Antabus was administered by stomach tube. At first, one dose of 0.5 g./kg. was given; however, there was no appreciable rise in blood acetaldehyde after intravenous injection of 3.5 ml. of 19 per cent alcohol in saline, as can be seen from Table 1, experiments 2 and 3.

TABLE 1

Figures represent mg acetaldehyde per 100 ml. blood

Expt. No.	After Alcohol: Initial (0 Minutes) Control	Initial (0 Minutes) Treated with Antabus	15 Minutes Control	15 Minutes Treated with Antabus	30 Minutes Control	30 Minutes Treated with Antabus	45 Minutes Control	45 Minutes Treated with Antabus
1	0.014	.....	0.057	.....	0.064	.....	0.036	.....
2*	.....	0.029	.....	0.064	.....	0.075	.....	0.054
3*	.....	0.018	.....	0.029	.....	0.025	.....	0.029
4	0.014	.....	0.122	.....	0.054	.....	0.178	.....
5	0.018	0.014	.....	0.450	0.050	0.528	0.054	0.482
6	0.024	.....	.....	.....	0.071	.....	0.075	.....
7	traces	0.018	0.054	0.086	0.043	0.228	0.036	0.203
8	.....	0.036	.....	0.154	.....	0.161	.....	0.129
9	traces	0.082	0.014	0.846	0.028	1.22	0.043	1.40
9a	.....	0.050	.....	0.461	.....	0.557	.....	0.718
10	.....	0.040	.....	0.429	.....	0.504	.....	0.475
11	0.068	0.082	0.146	0.846	0.139	1.22	0.131	1.40
11a	.....	traces	.....	0.332	.....	0.346	.....	0.607
12	0.050	.....	0.068	.....	0.068	.....	0.071	.....
13	0.025	0.089	0.061	0.375	0.089	0.753	0.150	0.768

* Only one dose of Antabus.

It was, therefore, decided to feed the pigeons Antabus[1] for 4–11 consecutive days, each time the dose being 0.5 g./kg. After this period, ethyl alcohol (10 ml. of 19 per cent kg.) was injected intravenously (alar vein), and

[1] Antabus (registered name) was kindly supplied by Messrs. Ayerst, McKenna, and Harrison Ltd., New York, N.Y.

the blood samples taken at intervals of 15, 30, and 45 minutes. An initial sample was taken prior to the injection of alcohol. As controls, in the majority of cases, the same pigeons were used, however, before being fed Antabus; the same amounts of alcohol were injected and the samples taken at the same time intervals. Acetaldehyde was determined by the method of Stotz[2].

Results

The results of our experiments are summarized in table 1. It may be noted from the initial values of acetaldehyde (prior to the injection of alcohol) that in pigeons on Antabus, the values were slightly higher than in the case of the controls (not fed Antabus). However, 15 minutes after injection of alcohol, the differences became much more manifested: the controls showed as highest values 0.122 mg. per cent and 0.146 mg. per cent acetaldehyde, whereas in the pigeons fed Antabus, acetaldehyde content of the blood rose in some cases up to 0.846 mg. per cent. After thirty minutes, the difference became in several cases even higher (up to 1.22 mg. per cent acetaldehyde) and was rising still after 15 more minutes, when the experiment was interrupted. It also is interesting to note that in cases where the control gave a relatively high value for acetaldehyde, as in experiment No. 11, the figures for the blank (after administration of Antabus but prior to the injection of alcohol) were correspondingly higher.

Discussion. From our results, it follows that in the pigeon, after the administration of tetraethylthiuram disulfide for several days, and after a single injection of ethyl alcohol, high amounts of acetaldehyde accumulate in the blood. These amounts which are comparable to those obtained in man, cannot be explained on the basis of xanthine oxidase inhibition as claimed by Westerfeld and his co-workers. In contrast to the rat and man, the pigeon liver is completely devoid of this enzyme and therefore, it becomes highly improbable that the *in vivo* activity of Antabus is based on this mechanism. According to Richert and Westerfeld, pigeon kidneys contain a small amount of xanthine oxidase, however, it does not seem

[2] We wish to acknowledge the technical help of Mrs. Dorotha Roark and Mr. Harry A. Roberts, Jr. We also thank Dr. Otto Bessey for reading the manuscript and Miss Leedia Greve for the preparation of it.

probable that these organs play an important part in the acetaldehyde metabolism. Lubin and Westerfeld demonstrated very clearly that the acetaldehyde metabolism is almost exclusively connected with the liver. We are therefore inclined to assume that Kjelgaard's point of view, that primarily the aldehyde oxidases in the liver are inhibited by Antabus, is valid *in vivo*. The high figures of acetaldehyde accumulation in the blood of pigeons fed Antabus support Kjelgaard's findings to a great extent.

Summary. Studies *in vivo* on the influence of tetraethylthiuram disulfide (Antabus) upon the alcohol metabolism of pigeons were carried out. Pigeons were fed tetraethylthiuram disulfide for several consecutive days, after which alcohol was injected intravenously and the accumulation of acetaldehyde in the blood estimated by the method of Stotz. The results show a steady increase in acetaldehyde contents of blood 15, 30, and 45 minutes after injection of alcohol. This increase, in extreme cases, reached as high as 1.40 mg. per cent (initial value before administering alcohol: 0.082 mg. per cent). As the pigeon liver does not contain xanthine oxidase, the conclusion was reached that *in vivo* other enzymes connected with the breakdown of acetaldehyde are inhibited by the tetraethylthiuram disulfide.

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THE EFFECT OF DIGITOXIN AND DIGOXIN ON CARDIAC RHYTHM AND ARTERIAL PLASMA POTASSIUM BEFORE AND AFTER EPINEPHRINE INJECTION IN UNANESTHETIZED DOGS*

G. S. O'Brien,† John Rockey, Quillian R. Murphy, Jr., and W. J. Meek‡

Since the clinical introduction of the potent purified digitalis glucosides, reports have appeared describing the occurrence of serious cardiac arrhythmias associated with their use (Levine, 1948; Fremont and King, 1950). In a few cases having a fatal outcome, death has been proved to be the result of ventricular fibrillation (Enselberg *et al.*, 1951).

Although digitalis glucosides long have been known to increase irritability of the mammalian myocardium, the degree to which this occurs may not be fully realized when electrocardiographic changes are minimal. There are a number of conditions, namely: inhalation of cyclopropane, chloroform (Meek, 1941) or benzol and administration of iminoazole derivatives (Dawes, 1952) and ouabain (Stutzman *et al.*, 1942), in which there is an increase in cardiac sensitivity yet the electrocardiogram alone may not provide such evidence. However, if a dose of epinephrine insufficient in amount to produce more than a few ventricular extrasystoles or a slow ventricular rhythm in the unanesthetized, unpremedicated dog is injected into the animal under one of these conditions, the increase in cardiac sensitivity is shown by a ventricular tachycardia.

The use of orally administered potassium salts to abolish ventricular arrhythmias of digitalis origin has been found effective

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† Predoctorate U.S. Public Health Fellow, 1950–1952, National Heart Institute.

‡ Emeritus Professor at Wisconsin and Lecturer at the Medical Branch, The University of Texas, Galveston.

in some patients (Sampson and Anderson, 1932; Sampson *et al.*, 1943; Enselberg, *et al.* 1950; Lown *et al.*, 1951). Castleden (1941), however, cautioned against the use of potassium salts to suppress cardiac arrhythmias in patients because of their unpredictable effects. In dogs it has been reported (Ginsburg, 1928; Cattell, 1943) that the serum potassium is elevated a small amount after rapid digitalization and that the heart is more adversely affected by the intravenous injection of potassium than normal (Camp, 1939).

It is known, also, that the intravenous injection of epinephrine can elevate arterial plasma potassium (D'Silva, 1936).

In view of the above findings, it seemed desirable to investigate: 1) the cardiac response to epinephrine in unanesthetized dogs previously given digitoxin and digoxin in such doses as to cause only minimal electrocardiographic changes, 2) the effect of these doses one hour after digitalization on the arterial plasma potassium level, and 3) the relationship of the cardiac response produced by epinephrine to alterations in arterial plasma potassium.

Methods. Trained, unanesthetized, healthy adult male and female dogs, weighing between 10.0 and 26.0 Kg., were used for these experiments. Food was withheld from the animals 18 to 24 hours prior to any of the procedures. Water was allowed *ad libitum* during the fasting period.

The cardiac response to epinephrine[1] was determined for each dog as previously described (Meek *et al.*, 1937). A control dose of epinephrine was selected which had been shown to produce, at most, a few seconds of slow ventricular rhythm. This was diluted to a volume of 5.0 cc. with isotonic glucose and injected intravenously at a constant rate of 1.0 cc. per 10 seconds. Electrocardiographic tracings from lead II were taken every 10 seconds during the injection and thereafter until the cardiac manifestations of epinephrine had subsided.

Twenty-one dogs received digitoxin[2] intravenously (.3 mg./Kg. body weight) and one hour later the cardiac effect of the control dose of epinephrine was determined. After an interval of at least four weeks, 15 of these animals were given digoxin[3] intravenously (.15 mg./Kg. body weight) and one hour later the cardiac response to the control dose of epinephrine was tested.

In 12 of these dogs arterial blood samples were taken for potassium analysis during the epinephrine injection before and after each of the digitalis

[1] Adrenalin Hydrochloride—Park, Davis & Co.

[2] Crystodigin—Lilly Laboratories. In part, kindly supplied by K. K. Chen, M.D.

[3] Digoxin—Burroughs, Wellcome & Co.

preparations. Blood withdrawals were made through an 18 or 20 gauge needle by direct puncture of the femoral artery through the skin. Blood samples of 4.0 cc. volume were withdrawn at a rate of 1.0 cc. per second into heparinized syringes before the injection of epinephrine and at intervals of 20, 40, 60, 70, 80, 100, 120, 180, and 300 seconds after the beginning of the epinephrine injection. These blood samples were centrifuged immediately, and the plasma was removed. The plasma was analyzed for potassium with an accuracy of ± 1.0 per cent using the Beckman flame spectrophotometer according to the method described by Mosher *et al.*, 1949.

Results

The results of the experiments are summarized in table 1. One hour after digitoxin and prior to epinephrine injection in 21 dogs there was noted SA rhythm in 17, A-V rhythm in 1, occasional ventricular extra-systoles in 2, and ventricular tachycardia in 1. With the injection of the control dose of epinephrine into the 20 of these dogs which had no ventricular tachycardia, there resulted ventricular tachycardia of 150 to 250 beats per minute in 11 animals, fast ventricular rhythm of 100 to 125 beats per minute in 2, slow ventricular rhythm of 37 to 84 beats per minute in 4, A-V block in 1, and A-V rhythm in 2. In dog No. 14 which showed ventricular tachycardia one hour after digitoxin, the injection of epinephrine produced no change in this arrhythmia.

The effect of digoxin was tested in 15 dogs. One hour after the administration of digoxin and prior to the epinephrine injection there was observed SA rhythm in 14 and ventricular extrasystoles in 1 of these animals. The injection of the control dose of epinephrine produced ventricular tachycardia of 150 to 250 beats per minute in 13 of these animals and a fast ventricular rhythm of 100 to 125 beats per minute in 2.

As can be seen in table 1, the arterial plasma potassium levels averaged 16.8 mg. per cent before digitalization in 17 dogs, 26.2 mg. per cent one hour after digitoxin in the same 17 animals, and 28.0 mg. per cent one hour after digoxin in 12 of these animals. The ranges were, respectively: 15.0 to 19.5 mg. per cent, 18.8 to 36.3 mg. per cent, and 18.0 to 32.0 mg. per cent. An intravenous injection of ethyl alcohol equivalent to that contained in the digitalis preparations produced no such potassium elevations. The maximum levels attained by the arterial plasma potassium with the subsequent injection of epinephrine in 12 dogs averaged 28.7 mg. per cent for the control observations, 33.8 mg. per cent

TABLE 1

Effect of Digitoxin and Digoxin on Cardiac Rhythm and Arterial Plasma Potassium Before and After Epinephrine in Unanesthetized Dogs

Dog No.	Before Digitalization			One Hour After Digitoxin (.3 mg./Kg. i.v.)				One Hour After Digoxin (.15 mg./Kg. i.v.)			
		During Epi. Inj.		Before Epi.		During Epi. Inj.		Before Epi.		During Epi. Inj.	
	Before Epi. K Level (mg. %)	Max. K Level (mg. %)	Cardiac Rhythm	K Level (mg. %)	Cardiac Rhythm	Max. K Level (mg. %)	Cardiac Rhythm	K Level (mg. %)	Cardiac Rhythm	Max. K Level (mg. %)	Cardiac Rhythm
1.....	...	...	VEX	...	SA	...	AVR	...	...	...	...
2.....	...	...	VEX	...	SA	...	VT	...	...	...	...
3.....	...	...	SVR	...	SA	...	SVR	...	...	...	...
4.....	...	...	SVR	...	AVR	...	VT	...	...	...	...
5.....	15.0	...	AVB	18.8	SA	...	FVR	...	...	...	...
6.....	17.2	...	VEX	24.8	SA	...	VT	...	...	...	...
7.....	15.0	25.3	SA	27.0	SA	37.0	FVR	32.0	SA	45.0	VT
8.....	16.6	...	AVR	22.6	SA	...	VT	...	SA	...	VT
9.....	16.5	...	AVR	26.7	SA	...	VT	...	...	...	...
10.....	16.3	...	VEX	30.8	SA	...	AVB	...	...	...	...
11.....	16.3	31.5	SA	27.0	SA	39.5	VT	31.3	SA	45.0	VT
12.....	18.3	26.0	VEX	23.0	SA	30.5	SVR	28.8	SA	32.5	FVR
13.....	18.0	35.5	AVR	23.3	SA	29.0	VT	23.3	SA	40.3	VT
14.....	15.5	28.0	SVR	18.8	VT	25.0	VT	18.0	SA	23.5	VT
15.....	16.3	24.3	VEX	23.5	VEX	26.3	VT	22.0	VEX	23.8	VT
16.....	17.0	29.3	SA	36.3	SA	45.0	AVR	29.3	SA	39.5	VT
17.....	16.3	29.8	VEX	24.0	SA	32.5	VT	27.0	SA	38.5	FVR
18.....	18.3	29.5	AVR	33.0	SA	40.5	VT	30.5	SA	40.8	VT
19.....	17.5	30.0	SA	22.0	SA	29.3	VT	23.0	SA	34.5	VT
20.....	19.5	30.3	SVR	30.0	SA	34.5	SVR	30.0	SA	35.8	VT
21.....	15.5	25.0	SA	33.3	VEX	36.0	SVR	28.3	SA	35.5	VT
Ave.....	16.8	28.7		26.2		33.8		28.0		36.2	

SA Sino-Auricular Rhythm.
AVR Auricular-Ventricular Rhythm—Rates Range 37 to 75 Beats per Minute.
AVB Auricular-Ventricular Block.
VEX Occasional Ventricular Extrasystole.
SVR Slow Ventricular Rhythm—Rates Range 37 to 84 Beats per Minute.
FVR Fast Ventricular Rhythm—Rates Range 100 to 125 Beats per Minute.
VT Ventricular Tachycardia—Rates Range 150 to 250 Beats per Minute.

after digitoxin, and 36.2 mg. per cent after digoxin. The ranges were: 24.3 to 35.5 mg. per cent, 25.0 to 45.0 mg. per cent, and 23.5 to 45.0 mg. per cent, respectively. It was noted that invariably the cardiac arrhythmias produced by epinephrine after digitoxin or digoxin first appeared during the rapid rise of potassium and continued during the period in which potassium was elevated by epinephrine.

The effect of epinephrine on arterial plasma potassium and cardiac rhythm before and after digitoxin and digoxin is shown in figure I for Dog No. 7. The changes noted in this figure were

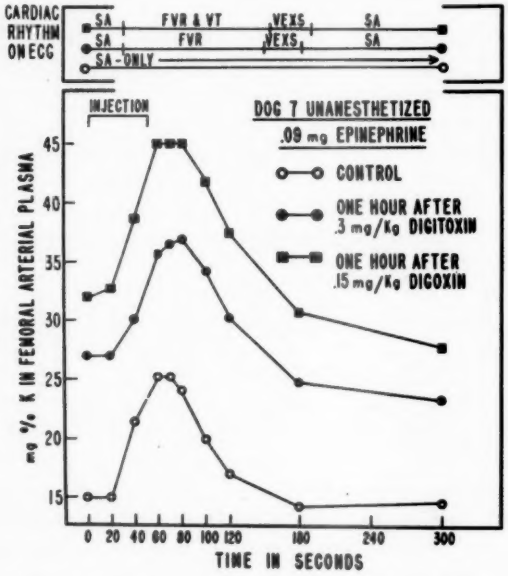

FIG. I. (Dog No. 7.) The effect of epinephrine on cardiac rhythm and arterial plasma potassium before digitalization and after digitoxin and digoxin. Each point on the graph indicates the potassium level at a time after the beginning of the epinephrine injections which were started at time zero. The arrhythmias and the time of their occurrence are shown on the horizontal lines at the top of the figure. SA, sinoauricular rhythm; VEX, ventricular extrasystoles; FVR, fast ventricular rhythm; SVR, slow ventricular rhythm; and VT, ventricular tachycardia.

typical for 9 dogs after digoxin in that the potassium rise during the epinephrine injection was abrupt and reached a maximum level considerably higher than the control, and fast ventricular rhythm or ventricular tachycardia occurred during this rise of potassium. In one dog the maximum level attained after digoxin was no greater than on the control yet ventricular tachycardia appeared. Figure I is characteristic for 6 dogs after digitoxin so far as potassium changes are concerned. Ventricular tachycardia appeared in 5 and a slow ventricular rhythm in one of these dogs during the rapid rise of potassium. Figure II (Dog No. 21) shows

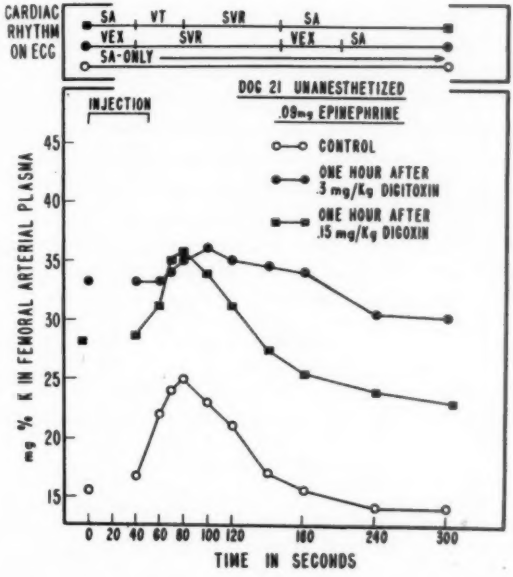

FIG. II. (Dog No. 21.) The effect of epinephrine on cardiac rhythm and arterial plasma potassium before digitalization and after digitoxin and digoxin. Each point on the graph indicates the potassium level at a time after the beginning of the epinephrine injections which were started at time zero. The arrhythmias and the time of their occurrence are shown on the horizontal lines at the top of the figure. SA, sinoauricular rhythm; VEX, ventricular extrasystoles; FVR, fast ventricular rhythm; SVR, slow ventricular rhythm; and VT, ventricular tachycardia.

a potassium response to epinephrine which was typical for 4 dogs after digitoxin. Here the increase in potassium is gradual. Ventricular tachycardia appeared in 1, slow ventricular rhythm in 2, and A-V rhythm in 1 of these animals. In only two dogs (Nos. 14 and 15) was the potassium level during epinephrine only slightly elevated when ventricular tachycardia occurred. In one of these animals ventricular tachycardia was present before the injection of epinephrine.

Summary and Conclusions. From these studies it is evident that in most dogs when unanesthetized, digitoxin and digoxin increased the sensitivity of the heart to an intravenous injection of epinephrine. One hour after digitoxin or digoxin the arterial plasma potassium was at a higher level than normal, and although the actual rise which followed a subsequent injection of epinephrine was slightly less than in the normal animal, the level reached was much higher in most animals. It may be considered that the potassium in these animals had now reached a critical level which possibly had an effect on the production of arrhythmias. It was noted invariably that the cardiac arrhythmias produced by epinephrine after digitoxin or digoxin were definitely related in time to the elevation of plasma potassium produced by the epinephrine.

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ELECTRON MICROGRAPHS OF ERYTHROCYTES FROM SWISS ALBINO MICE INFECTED WITH YELLOW FEVER VIRUS (STRAIN 17D)*

REGINALD L. REAGAN, MILDRED T. STEWART, AND ARTHUR L. BRUECKNER

The 17D strain of yellow fever virus used for this study was furnished by the American Type Culture Collection of Washington, D.C. This virus had been obtained originally from Dr. Sawyer of the Rockefeller Foundation Laboratories. The 229th passage of the 17D strain was received at the Rocky Mountain Laboratory from the Rockefeller Foundation as Lot 145–3 AB 769. The strain has been through five passages in embryonated eggs at the Rocky Mountain Laboratory. The 17D strain is widely used for immunization of man against yellow fever. It produces no viscerotropic lesions in man or monkey when inoculated subcutaneously but induces a mild systematic disease with small amounts of circulating virus (Theiler, 1930).

Materials and Methods. The virus received here was lyophilized chick embryo tissue and was diluted to a 10 per cent suspension with physiological saline. Fifteen female Swiss Albino mice (3 weeks old) were inoculated intracerebrally with 0.03 ml. of this virus suspension of the 5th egg embryo passage. The mice showed nervous symptoms of involuntary motor reactions and paralysis on the 7th day. When symptoms appeared the mice were sacrificed, and the brains and cords were removed asceptically, ground in a mortar with alundum, and diluted to a 20 per cent suspension with sterile physiological saline. This material was used as inoculum for the present experiment. The present experiment was conducted in the following manner: 28 healthy female Swiss Albino mice (21 days old), Webster and Dawson, 1935, were divided into two groups of fourteen animals each. One group was designated as the control group and the other as the test group. One-tenth ml. of a 20 per cent suspension of the above described inoculum was injected intraperitoneally into each of the fourteen Swiss Albino mice in the test group. Animals of the control group were inoculated intraperitoneally with 0.1 ml. of a 20 per cent brain suspension prepared from normal Swiss Albino mice of the same strain and age as previously mentioned. The animals of each group were further divided into seven groups of two animals each. Two animals from the test group and two animals from the control group were bled from the heart every 24 hours post inoculation,

* From the Virus Laboratory, Livestock Sanitary Service, University of Maryland, College Park, Maryland.

drawing approximately 0.1 ml. of blood from each animal. The blood taken from the two animals in the test group was pooled and added to 1 ml. of heparinized physiological saline. The blood from the two animals of the control group was treated in the same manner. The erythrocyte suspension from each group was then further diluted by adding 1 ml. of physiological saline to each specimen. The cell suspension from each group was then employed for electron microscopic studies. The same procedure was carried out on erythrocyte suspensions taken from mice of the test and the control groups at time intervals of 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, 168 hours, and 192 hours post inoculation. Only animals from the test group at the 168 hours interval exhibited symptoms of central nervous system involvement such as that found in mice infected intracerebrally with this virus strain. The mice from the control group showed no symptoms of nervous system involvement.

Each specimen was prepared for electron microscopic examination by placing small drops of the erythrocyte-heparinized saline suspension on parlodion film supports which had been prepared 90 hours previously. The films were dried and shadowed with chromium. William and Wyckoff, 1946, at arc tangent 4/12 and examined under the R.C.A. electron microscope, type E.M.U.

In order to determine the infectivity of the erythrocytes obtained from animals of the test group, 28 mice were divided into seven groups of four mice each, and the mice from each group were inoculated intracerebrally with 0.03 ml. of an erythrocyte suspension from a designated time interval. In order to determine the presence of any toxic material in the diluent (heparinized saline) each of four normal Swiss Albina mice was inoculated intracerebrally with 0.03 ml. of an erythrocyte-heparin-saline suspension. The erythrocyte specimen was taken from the mice of the 72 hour control group. After inoculation the mice were observed twice daily over a 21-day period.

Results

Upon examination by electron microscopy of the erythrocytes taken at designated time intervals from the control group, no virus-like particles could be detected as shown in figure VIII and figure IX. However, upon examination of the erythrocyte suspension taken from the infected Swiss Albino mice, a few virus-like particles were seen on the surface and outside of the erythrocytes obtained at the following time intervals: 24 hours (Fig. Ia and Ib), 48 hours (Fig. II), 72 hours (Fig. III), 96 hours (Fig. IVa and IVb), 120 hours (Fig. Va and Vb), 168 hours (Fig. VIa and VIb). These virus-like particles had the same shape and size as those demonstrated by the electron microscope of the yellow fever virus (strain 17D) from infected mouse brains, Reagan

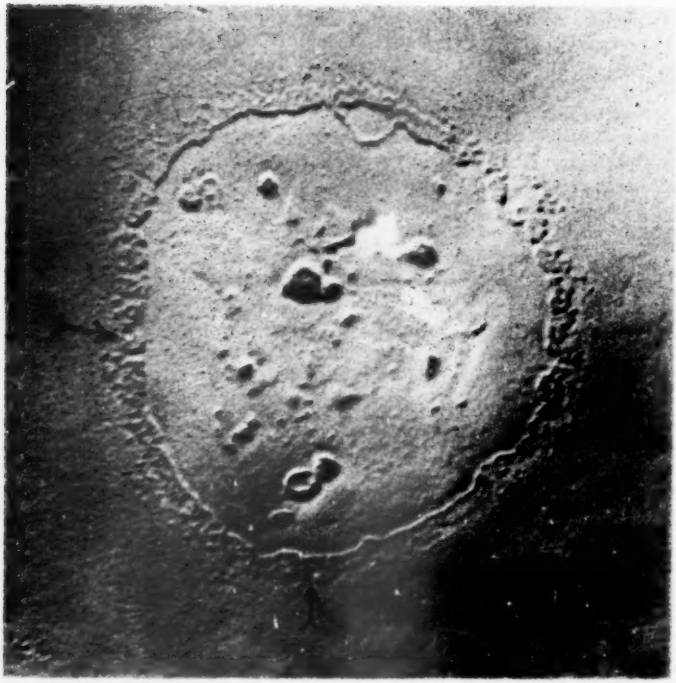

FIG. I-a. Erythrocytes from mouse infected with yellow fever (17D strain). Time interval—24 hrs. Shadowed with chromium arc Tangent 4/12, X 32,000. Virus-like particles indicated by arrows.

FIG. I-b. Erythrocytes from mouse infected with yellow fever (17D strain). Time interval—24 hrs. Shadowed with chromium arc Tangent 4/12, X 32,000. Virus-like particles indicated by arrows.

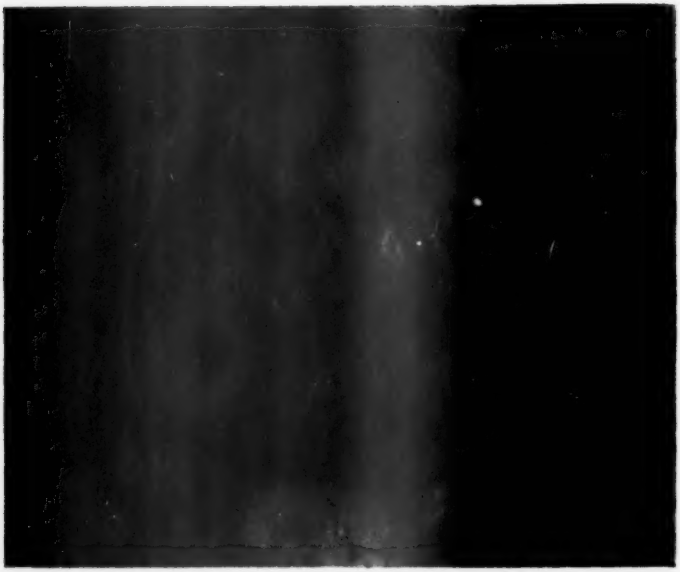

FIG. II. Erythrocytes from mouse infected with yellow fever (17D strain). Time interval—48 hrs. Shadowed with chromium arc Tangent 4/12, X 32,000. Virus-like particles indicated by arrow.

FIG. III. Erythrocytes from mouse infected with yellow fever (17D strain). Time interval—72 hrs. Shadowed with chromium arc Tangent 4/12, X 32,000. Virus-like particles indicated by arrow.

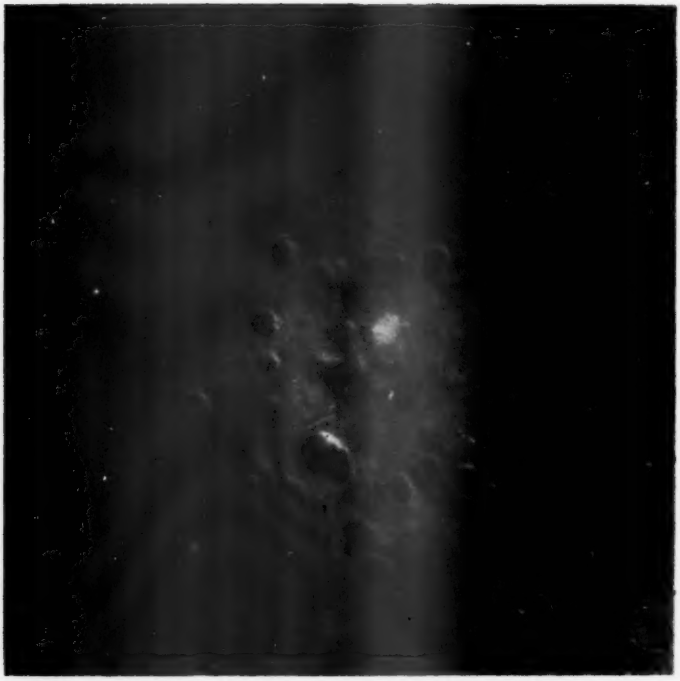

FIG. IV-a. Erythrocytes from mouse infected with yellow fever (17D strain). Time interval—96 hrs. Shadowed with chromium arc Tangent 4/12, X 32,000. Virus-like particles indicated by arrow.

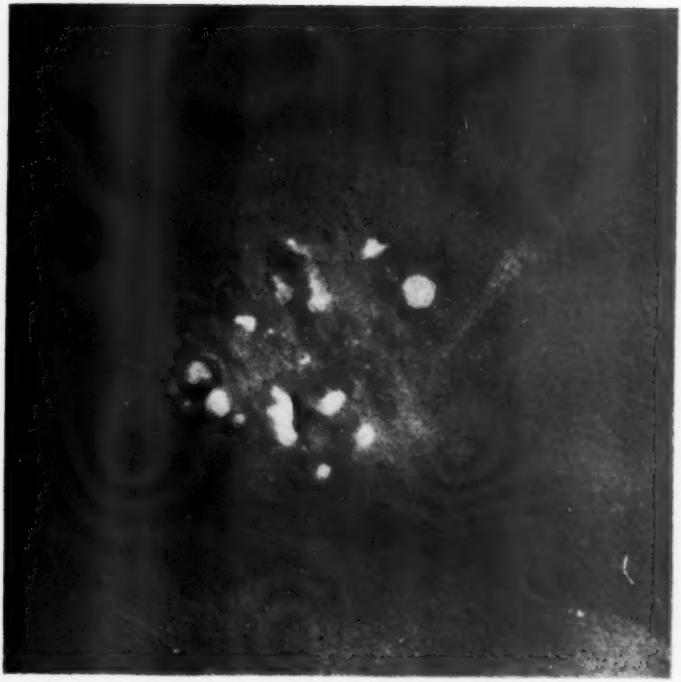

FIG. IV-b. Erythrocytes from mice infected with yellow fever (17D strain). Time interval—96 hrs. Shadowed with chromium arc Tangent 4/12. X 32,000. Virus-like particles indicated by arrow.

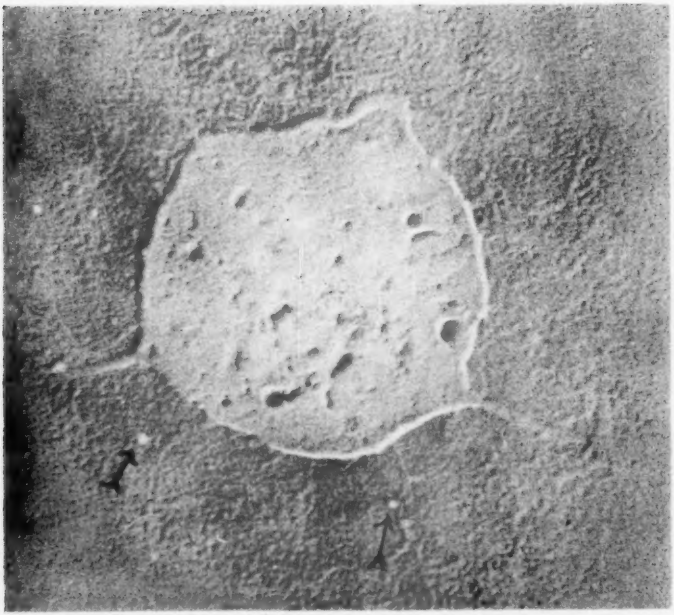

FIG. V-a. Erythrocytes from mouse infected with yellow fever (17D strain). Time interval—120 hrs. Shadowed with chromium arc Tangent 4/12, X 32,000. Virus-like particles indicated by arrows.

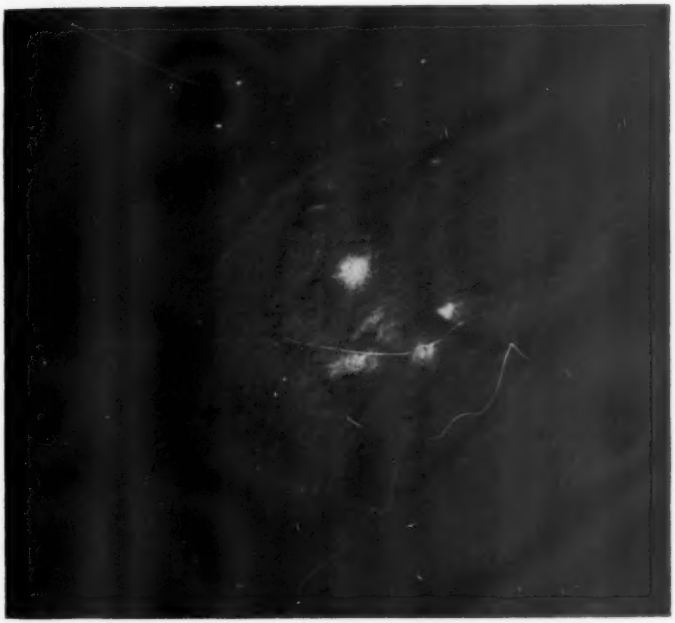

FIG. V-b. Erythrocytes from mouse infected with yellow fever (17D strain). Time interval—144 hrs. Shadowed with chromium arc Tangent 4/12, X 32,000. Virus-like particles indicated by arrows.

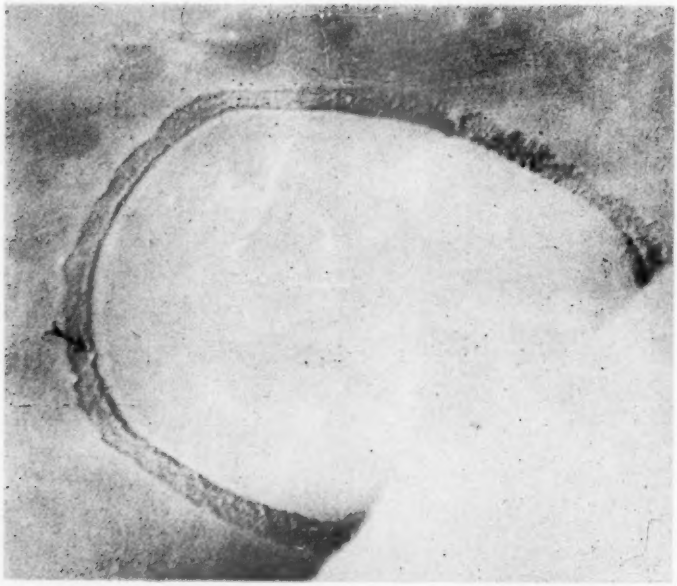

FIG. VI-a. Erythrocytes from mouse infected with yellow fever (17D strain). Time interval—16 hrs. Shadowed with chromium arc Tangent 4/12, X 32,000. Virus-like particles indicated by arrow.

FIG. VI-b. Erythrocytes from mouse infected with yellow fever (17D strain). Time interval—168 hrs. Shadowed with chromium arc Tangent 4/12, X 32,000. Virus-like particles indicated by arrows.

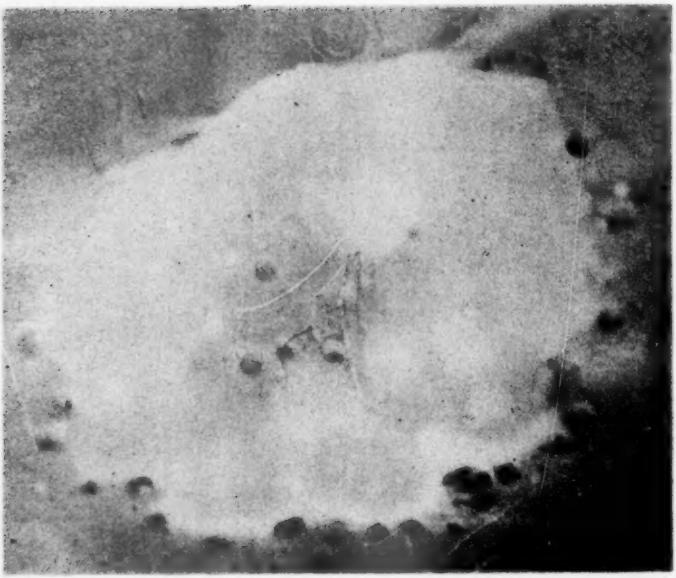

FIG. VII. Erythrocytes from mouse infected with yellow fever (17D strain). Time interval—192 hrs. Shadowed with chromium arc Tangent 4/12. X 32,000.

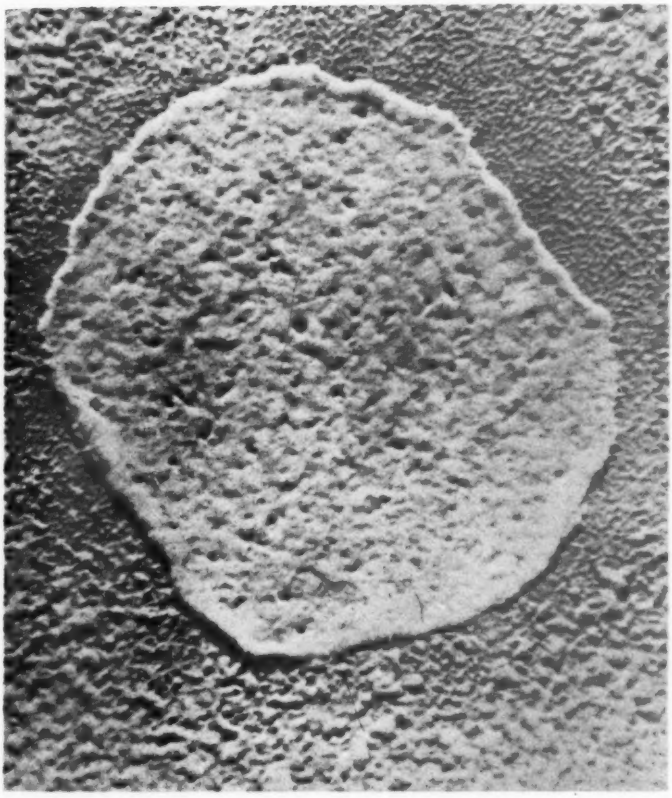

FIG. VIII. Erythrocytes from normal mouse. Time interval—72 hrs. Shadowed with chromium arc Tangent 4/12, X 32,000.

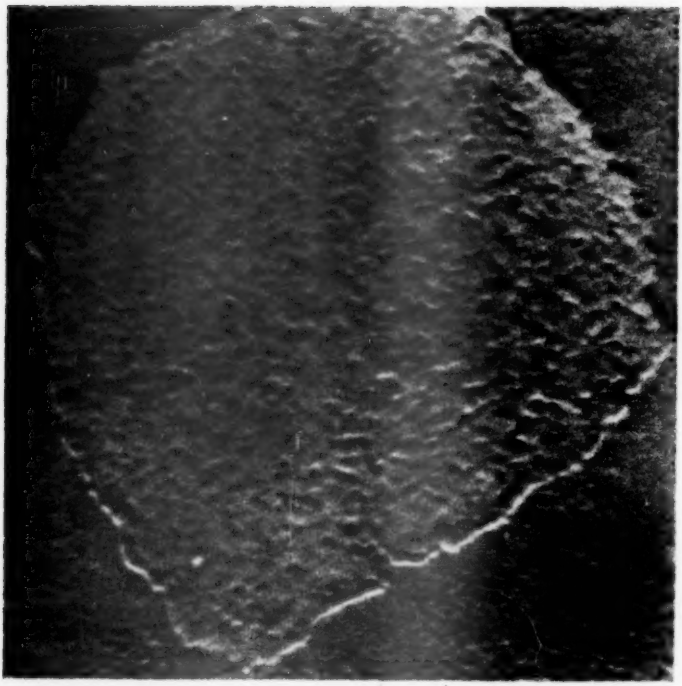

FIG. IX. Erythrocytes from normal mouse. Time interval—96 hrs. Shadowed with chromium arc Tangent 4/12. X 32,000.

and Brueckner, 1935. Electron micrograph made of erythrocytes taken from animals of the test group at the time interval of 192 hours (Fig. VII), did not show the presence of any virus-like particles.

Table 1 shows the infectivity of erythrocyte specimens from the test group when injected intracerebrally into Swiss Albino

TABLE 1

Response of Swiss Albino mice injected with Erythrocytes suspension from Swiss Albino mice infected with Yellow Fever Virus (17D strain)

Bleeding interval hours	No. of mice inoculated with erythrocytes	No. of mice showing symptoms	Min. to max. incubation* period, days
24	4	4	8
48	4	4	8
72	4	4	6–12
96	4	4	6–8
120	4	4	6–7
144	4	4	6–7
168	4	4	6–10
192	4	0	0
72 (normal)	4	0	0

* Time elapsing between inoculation and onset of symptoms.

mice. Those mice succumbing to the disease developed symptoms of central nervous system involvement within 6–12 days after inoculation. All four mice inoculated from the control group (72 hour) remained normal throughout the observation period. All surviving mice from control and test group were discarded after a 21 day observation period.

Discussion. From the results obtained in this experiment it was apparent that the virus was present in the blood stream from 24 to 168 hours post inoculation. The virus apparently left the blood stream between the 168 and 192 hour intervals. Electron micrographs of the virus-like particles showed the particles to be present on the surface or around the surface of the erythrocyte. These virus particles resemble the yellow fever virus (17D strain) in diameter and contour described by Reagan and Brueckner, 1935. In the 192 hour specimen no virus-like particles could be demonstrated by electron microscopy, and mice injected intracerebrally of this bleeding interval showed no sign of central nervous system involvement.

Summary. Electron microscopic studies were made of erythrocytes from Swiss Albino mice injected intraperitoneally with yellow fever virus (17D strain). Electron micrograph of erythrocytes obtained from infected mice at 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, and 168 hours post inoculation revealed the presence of virus-like particles resembling those of the yellow fever virus in shape and contour. The 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, and 168 hours, erythrocytes specimens were also infective for Swiss Albino mice when injected intracerebrally. No virus-like particles could be demonstrated in the 192 hours specimen, and the blood specimen was not infective for mice by the intracerebral route. Erythrocytes taken from the mice of the control group shows no virus-like particles on or around their surface. The same procedure was used for the control group as was used for the test group throughout the experiment.

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A NEW SPECIES OF *HIRSTIONYSSUS* AND A KEY TO THE KNOWN SPECIES OF THE WORLD

R. W. STRANDTMANN* AND HARVEY B. MORLAN†

Further basic biological knowledge is needed to elucidate the epidemiology of such rodent-borne diseases as plague and tularemia. The Communicable Disease Center has taken a step toward this objective by the establishment of a field ecology study at Santa Fe, New Mexico. One deterrent to the accumulation of significant ecological data has been the lack of names for many of the rodent ectoparasites. The following review and summary of a group of related species may clarify the taxonomic status of one new parasitic mite and contribute a source of facts useful to future studies.

Genera are subjective rather than objective units. We know of no universally accepted means or criteria for establishing a genus, nor for deciding what shall be a subgenus. It is our opinion that, especially during a time when new species of mites are being described at an accelerated rate, it is undesirable to lump groups with clearly recognizable differences into a single genus that may all too soon become unwieldy by reason of excessive numbers. We agree that *Neoichoronyssus*, *Patrinyssus*, and *Hirstionyssus* are closely related; however, their differences are of sufficient importance that we consider them as genera of equal rank, rather than following Jameson (*P. Ent. Soc.*, Wash., 52: 161, 1950) in designating them as subgenera of *Neoichoronyssus*. These genera may be characterized as follows:

Neoichoronyssus Fonseca, *Ciencia*, 2: 262, 1941–1942

Coarse, well sclerotized mites. Female with a spur ventrally on the basal palp segment, the genitoventral plate pointed posteriorly and some of the coxal spurs setigerous. The male ventral plate undivided and not dilated posterior to leg IV. Two species are known.

Neoichoronyssus wernecki (Fonseca), 1935. *Mem. Inst.*, Butantan, 9: 43. No tooth-like projections on any leg segment beyond the coxa, coxa I with a setigerous spur, coxae II, III, and IV devoid of spurs. Found on New World marsupials (Didelphiidae).

* Texas Technological College, Lubbock, Texas.
† Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Santa Fe, New Mexico.

Neoichoronyssus dentipes (Strandtmann and Eads), 1947, *J. Parasit.*, 33: 51. Tooth-like projections on several leg segments; spurs on all coxae. Found on cotton rats, *Sigmodon hispidus*, U.S.A.; also one specimen from *Proechimys* sp., Colombia.

Patrinyssus Jameson, 1950, *Proc. Ent. Soc.*, Wash., 52: 162

Heavily sclerotized mites with many prominent spurs and spines. Female lacking spurs on the basal palp segment: genitoventral plate rounded posteriorly, some of the coxal spurs setigerous. The *male ventral plate is divided* just behind the fourth coxae. Monotypic.

Patrinyssus hubbardi (Jameson), 1949, *J. Parasit.*, 35: 109. On the mountain beaver, *Aplodontia rufa*.

Hirstionyssus Fonseca, 1948, *P. Zool. Soc.* Lond., 118: 266

In general, delicate and weakly sclerotized mites. No palpal spurs in the female, genitoventral plate rounded posteriorly, dorsal plate tapering abruptly posteriorly, at least some of the coxae with spines beside the anterior one on coxa II, but none of these setigerous. Ventral plate of male undivided and very slightly dilated posterior to fourth pair of legs. Contrary to Prof. Zumpt's statement (*Moçambique*, 67: 6, 1951) the tritosternum is not lacking, although it may be poorly sclerotized.

Discussion. Species of *Hirstionyssus* are for the most part parasites of rodents. Of the 24 species of which females are known, only one, *staffordi*, is restricted to non-rodent hosts (skunks). Two others, *arcuatus* and *carnifex*, are found on Chiroptera as well as rodents, and *isabellinus* has been recorded from a skunk as well as various rodents. *Arcuatus* and *occidentalis* have also been recorded from shrews, *Sorex* spp.

Like most Macronyssid mites, these probably spend most of their time in the nest, crawling on the host only to feed and then retiring again to the nest. Only rarely does one find very large numbers on the host, but collections from nest material by one of us (HBM) produced these mites in quantity.

Immature stages are infrequently found, perhaps because they are of short duration, but both proto- and deutonymphal stages are known for some of the species. As far as we are aware, no one has ever worked out the life cycle of any mite of this genus, but it is probable that they are ovoviviparous. Indeed, for one species, *staffordi*, protonymyphal stages have been found within the female.

Detailed life histories of these mites are certainly in order. They are indubitably blood suckers and certainly are potential vectors and reservoirs of diseases of animals transmissible to man.

Key to the Known Species of *Hirstionyssus*, Based on the Females. The key to the females given below is based upon the actual specimens when possible, but where these were unavailable, the characters were drawn from the published descriptions and illustrations.

When such a key as this is attempted, it is especially exasperating to come across vague and very generalized descriptions with incomplete or inaccurate sketches. Oudemans' best papers might well be a model for all describers to emulate. His descriptions are complete and the drawings show not only the whole mite but many detailed enlargements of critical areas.

We do not wish to claim that the characters we have stressed are the final answer to species differentiation in this genus; they were used because of convenience and ease of finding. It may well be that future study will show that some of the characters here used are of no particular importance.

1. Without a pair of claw-like setae at the apex of tarsus II—2.
With a pair of such modified setae at the apex of tarsus II—12.

2. With three spurs on coxa II. (The dorsal anterior spur always included when we mention the spurs of coxa II.)—3.
With less than three spurs on coxa II—8.

3. With a spur on coxa IV; coxal spur pattern 0–3–2–1. On coxa II the marginal seta is replaced by a heavy spur bent at right angles. From "squirrels," Germany. (Fig. 1)—*pauli.*

Willmann, 1952. (Z. f. *Parasitenkunde*, 15: 407)

Coxa IV without a spur—4.

4. Sternal plate from one-half to two-thirds as long as wide—5.
Sternal plate narrow, arched, always more than three times as wide as long—6.

5. Sternal plate about one-half as long as wide; coxal spurs narrow, long, sharp. "Squirrels," France. (Fig. 2)—*sciurinus.*

Hist, 1921. (*P. Zool. Soc.*, Lond., p. 785)

Sternal plate about two-thirds as long as wide; coxal spurs broader, shorter. *Geomys*, U.S.A. (Fig. 3)—*geomydis.*

Keegan, 1946 (*Tr. Am. Micr. Soc.*, 65: 59)

6. Setation normal, rather delicate—7.
Dorsal and ventral setae all uniformly long and prominent. Peritreme long and unusually wide. Dorsal plate narrowly acuminate posteriorly; from *Dipodomys merriami*, U.S.A. (Fig. 4)—*triacanthus.*

Jameson, 1950. (*P. Ent. Soc.*, Wash., 52: 166)

7. Sternal plate very narrow, about eight times as wide as long. Peritremes extending to middle of coxa I or beyond. From *Dipodomys ordi*, *D. spectabilis* and *Onychomys leucogaster*, U.S.A. (Fig. 5)—*incomptus.*

Eads and Hightower, 1952. (*P. Ent. Soc.*, Wash., 54: 32)

Sternal plate about four or five times as wide as long. Peritremes extending only slightly beyond middle of coxa II. From *Perognathus parvus* and *P. flavus*, U.S.A. (Fig. 6)—*hilli.*

Jameson, 1950. (*P. Ent. Soc.*, Wash., 52: 165)

8. Coxa II with only the dorso-anterior spur. Spur pattern 0–1–2–1. From nests of *Neotoma albigula*, U.S.A. (Figs. 24–31)—*breviseta*, n. sp.
Coxa II with two spurs—9.

9. Coxa II and coxa III each with two spurs—10.
Coxa III with one spur. Spur pattern 0–2–1–1. Ventral setae fairly heavy. The laboratory mouse, *Mus musculus albinus*, Brazil. (Fig. 7)—*butantanensis.*

Fonseca, 1932. (*Mem. Inst.*, Butantan, 7: 136)

10. Coxa IV with a spur. Spurs on coxa II and III acute. From *Neotoma micropus, N. albigula,* and *N. fuscipes,* U.S.A. (Fig. 8)—*neotomae.*
Eads and Hightower, 1951. (*P. Ent. Soc.*, Wash., 53:293)

Coxa IV lacking a spur—11.

11. Inner spurs on coxae II and III rounded, blunt. From *Sorex trowbridgei;* also *Peromyscus, Neürotrichus,* and *Clethrionomys,* U.S.A. (Fig. 9) *obsoletus.*
Jameson, 1950. (*P. Biol. Soc.*, Wash., 63: 31)

Inner spurs on coxae II and III acute. Posterior margin of genitoventral plate, and the entire margin of the anal plate somewhat thickened. From *Arvicola amphibius, Mus musculus, Mustela erminea,* and *Talpa europea* in Europe. From *Microtus pennsylvanicus, M. montanus* and *Lagurus curtatus* in Canada and U.S.A. (Fig. 10)—*isabellinus.*
Oudemans, 1913. (See *Archiv. f. Naturg.* 79 [9]: 80)

12. Coxval spur pattern 0–1–1–0 (in all cases, the prominent anteriodorsal projection of coxa II is considered as a spur). Sternal plate about twice as wide as long. From "Alpine marmot," Europe; also *Cynomys ludovicianus,* England (Fig. 11)—*blanchardi.*
Trouessart, 1904. (See Hirst, 1921, *P. Zool. Soc., Lond.*, p. 787)

Coxal spur pattern other than above—13.

13. Coxa I with a spur—14.

Coxa I without spurs—16.

14. Coxa I with a huge spur. Coxal spur pattern 1–2–0–0. Tarsus II strongly hooked at apex and probably having two modified setae although the description says nothing of it. Sternal plate about four times as wide as long. No mention is made of the peritreme. From the Springhare, *Pedetes cafer.* Africa. (Fig. 12)—*santos-diasi.*
Zumpt, 1951. (*Moçambique,* 67: 3)

Coxa I with a spur of medium size. Peritreme short, barely reaching coxa II—15.

15. Coxal spur pattern 1–4–2–1. The posterior spine on coxa II is enormous. Sternal plate about twice as wide as long. From young porcupine. Africa. Said to have "simply swarmed on the porcupine, and the native who skinned it complained that they bit or stung him like a bee, but left no mark." (Fig. 13)—*creightoni.*
Hirst, 1912. (Bull. *Ent. Res.*, 3: 369)

Coxal spur pattern 1–3–2–1. The ventral spurs on II and III are bifid. Sternal plate with only 4 setae. From skunks, *Mephitis, Spilogale,* U.S.A. (Fig. 14)—*staffordi.*
Strandtmann and Hunt, 1951. (*J. Parasit.*, 37: 460)

16. Coxa II with four spurs, the inner anterior spur of coxa II being very large. Tarsus II with one tremendous and one smaller apical claw-like seta. From *Heliosciurus rufobrachium,* Africa. (Fig. 15)—*liberiensis.*
Hirst, 1912. (*B. Ent. Res.*, 3: 371)

Coxa II with two spurs—17.

17. No spur on coxa IV. Spur pattern 0–2–2–0—18.

Coxa IV with a spur. Spur pattern 0–2–2–1—19.

18. Peritreme short, extending only to middle of coxa II. The inner coxal spurs rounded at apex. From *Tamiasciurus douglasi;* also one record each from *Peromyscus boylii* and *Eutamias minimus,* U.S.A. (Fig. 16)—*affinis.*
Jameson, 1950. (*P. Ent. Soc.*, Wash., 52: 163)

Peritreme extending to coxa I, inner coxal spines long and acute. From *Spalax monticola,* Macedonia. (Fig. 17)—*macedonicus.*
Hirst, 1921. (*Proc. Zool. Soc. London, London:* p. 790)

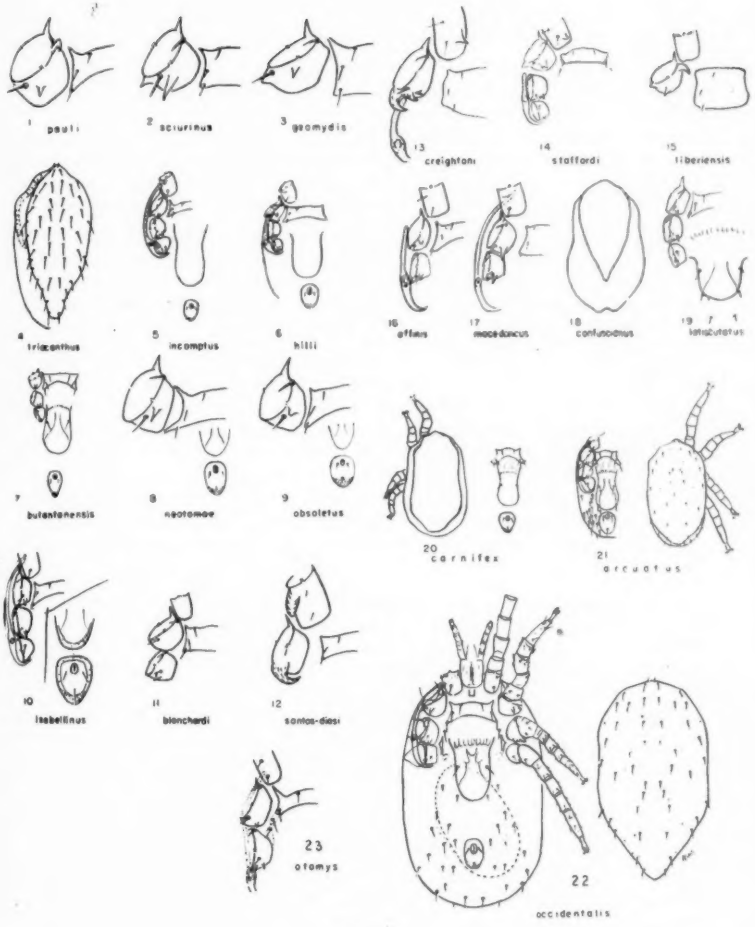

PLATE I

Hirstionyssus, diagnostic characters of the female.

19. Dorsal plate triangular. Inner coxal spurs blunt. From *Myospalax cansus*, North China. (Fig. 18)—*confuscianus.*

Hirst, 1921. (See Hirst, 1921, *P. Zool. Soc.*, Lond., p. 364 and p. 789)

Dorsal plate elliptic, although the posterior tip may be roundly acuminate—20.

20. Legs noticeably short and stubby, dorsal plate concave laterally. From *Vespertilio* sp., also *Rattus* spp., *Microtus arvalis, Peromyscus* spp., *Pterygistes noctula.* Cosmopolitan. (Fig. 20)—*carnifex.*

C. L. Koch, 1839. (See Oudemans, 1913. *Arch. f. Naturg.*, 79: 75)

Legs longer, more slender. Lateral margins of dorsal plate straight or convex—21.

21. Ventral setae delicate. Anal and genitoventral plate separated by much more than the diameter of the anal pore—22.

Ventral setae rather heavy, coxal spurs long, acute; anal and genitoventral plates separated by less than the diameter of the anal pore. From *Vespertilio noctula;* also *Rattus rattus, Mus musculus, Talpa europea, Mustela* sp., *Myotis* spp., *Eptisicus serotinus, Pterygistus noctula,* Europe: also "Rat," Japan: also *Blarina brevicauda, Cryptotis parva, Microtus pennsylvanicus, Neürotrichus gibbsi, Parascalops breweri, Sorex cinereus,* and *Sorex fumeus,* U.S.A. The specimens from *Sorex fumeus* have the anterior marginal seta of coxa II very short and heavy. (Fig. 21)—*arcuatus.*

C. L. Koch, 1839. (See Oudemans, 1913. *Archiv. f. Naturg.*, 79: 68)

22. Genitoventral plate quite short, about as broad as long, and seeming to originate at about the middle of the fourth coxae. From *Rattus rattus,* Africa. (Fig. 19)—*latiscutatus.*

Meillon and Lavoipierre, 1944. (*J. Ent. Soc.*, S. Africa, 7: 38)

Genitoventral plate longer than wide, originating anterior to coxa IV—23.

23. Sternal plate completely sclerotized; endopodal seta placed opposite the middle of coxa III, or very nearly so. From "mouse," white mice, *Citellus richardsoni elegans, C. beldingi, Aplodontia rufa, Glaucomys sabrinus, Peromyscus boylii, P. leucopus, P. maniculatus, Rattus rattus, Sorex trowbridgei. Lepus californicus,* and *Cynomys* sp., U.S.A. (Figs. 22 and 32–35)—*occidentalis.*

Ewing, 1922. (*P.U.S. Nat. Mus.*, 62: 20)

Sternal plate very narrow, its posterior angles poorly sclerotized so that it appears to bear only four setae; endopodal seta placed midway between coxa III and coxa IV. From the water rat, *Otomys* sp., Africa—*otomys.*

Radford, 1942. (*Parasit.*, 34: 190)

Hirstionyssus breviseta n. sp.

Female. (Figs. 24, 25, and 26) Length of body, exclusive of gnathosoma, 440 μ.; width 280 μ. Sternal plate faintly reticulated, about three times as wide as long, anterior margin nearly straight, lateral and posterior margins concave, with three pairs of subequal setae and the usual two pairs of pores. Tritosternum delicate, pilose from point of bifurcation, reaching to base of pedipalp trochanter. Genitoventral plate broadly rounded posteriorly, very slightly expanded below genitoventral pair of setae. Distance between the genitoventral and anal plates greater than the length of the anus. Anal plate pyriform, anus near anterior margin of plate, paired setae inserted near posterior margin of anus, with cribrum posterior to postanal seta which is equal to, or only slightly larger than, the paired setae.

Uncovered venter striate, with about 17 pairs of setae in addition to the endopodal setae. One pair of setae appear at first glance to be on the genitoventral plate, but the point of insertion is about half encircled by an indentation in the caudolateral margin of the genitoventral plate. Stigma at a level between coxae III and IV, peritreme extending anteriorly almost to the middle of coxa I and caudally contiguous with the parapodal plate.

Dorsal plate widest at about the level between legs III and IV, narrowed posteriorly to a broadly rounded tip, with fine net-like reticulation, with about 26 pairs of short, fine setae and about 15 pairs of tiny pores. Setae on posterior margin are slightly larger than the anterior and median setae of the plate. Uncovered dorsum striate, with about 12 pairs of setae.

Legs I and II slightly heavier than legs III and IV. Tarsus II with slender setae, lacking a pair of claw-like spines. Coxa I with two slender setae. Coxa II with two slender setae and the usual dorsal anterior spur. Coxa III with two slender setae and two acute spurs. Coxa IV with an anterior seta and a short, acute spur near posterior margin.

Gnathosoma: Arms of chelae are slender, about equal in length (Fig. 25). Movable arm heavier, tapered from distal one-third to a subacute tip. Fixed arm curved, tapered distally to a delicate, slightly expanded tip which is easily overlooked. In some specimens it is possible to observe a short, weak pilus dentilus inserted on the distal half of the fixed arm. Tectum (epistome) tapered distally, reaching to end of fourth segment of palpus; anterior margin with short, acute, median projection (Fig. 26). Hypostome with about 14 teeth, mainly alternated in a single row. With the usual four pairs of hypostomal setae; basal pair longest; of the middle pairs, the outer setae are comparatively tiny.

Male, (Figs. 27, 28, and 29) Length of body, exclusive of gnathosoma, 330 μ; width 210μ. Holoventral plate faintly reticulated, broadest anteriorly; with lateral projections between coxae I and II, II and III, and III and IV; slightly expanded posterior to coxae IV and constricted anterior to anus. Paired anal setae, near anterior margin of anus, subequal to unpaired seta inserted just anterior to cribrum; eight other pairs of setae and three or four pairs of pores on holoventral plate. Uncovered venter striated, with about 19 pairs of setae. Stigma, peritreme, tritosternum, and coxae similar to female. Legs similar to female except for the presence of claw-like spines ventrally on apex of tarsus II.

Dorsal plate reticulated, with about 26 pairs of short, fine setae and about 13 pairs of pores; posterior pair of setae are longest. The posterior margin of the dorsal plate is more broadly rounded than in the female. The narrow band of uncovered dorsum has about nine pairs of marginal setae.

Chelae heavier than in female; movable arm rounded apically, longer and heavier than fixed arm which has an acute apex and delicate pilus dentilus. Other structures of the gnathosoma appear similar to female.

Deutonymph. (Figs. 30 and 31) Length, varies from 300–400 μ; width from 190–280 μ. Sternal plate broadest anteriorly, tapering to a narrow posterior margin below coxae IV, with four pairs of setae and three pairs of

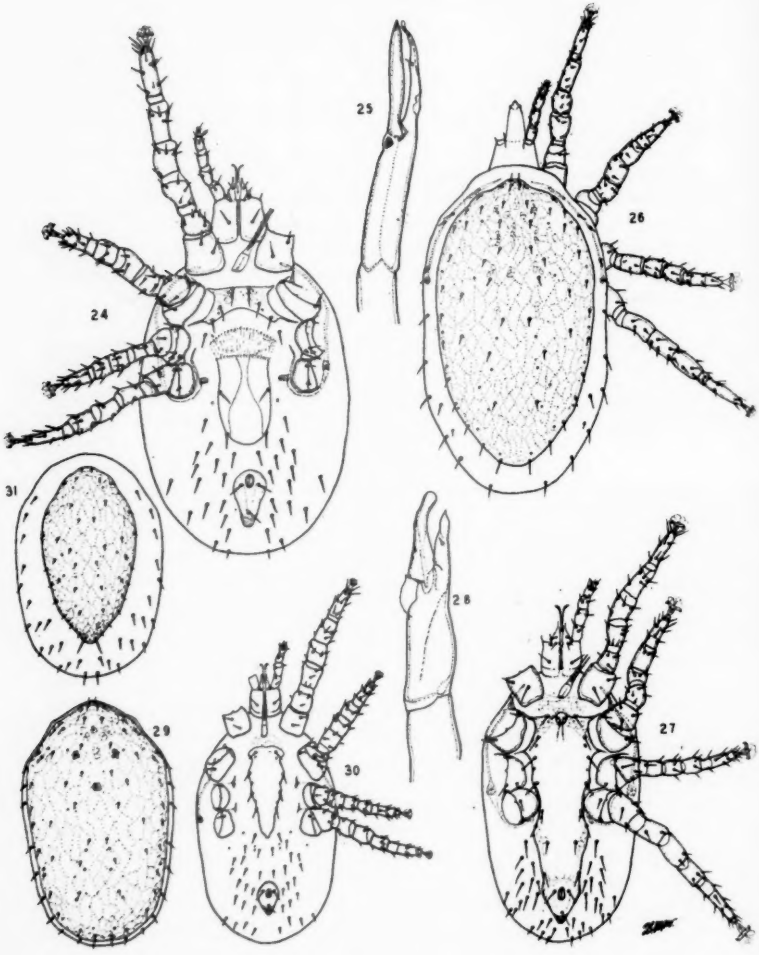

PLATE II

Hirstionyssus breviseta, n. sp.: Figure 24, ventral view of female; 25, female chelicera; 26, dorsal view of female; 27, ventral view of male; 28, male chelicera; 29, dorsum of male; 30, ventral view of deutonymph; 31, dorsum of deutonymph.

pores. Anal plate pyriform, with the usual three, about equal setae, and distinct cribrum. Uncovered venter striated, with several tiny platelets posterior to coxae IV and about 18 pairs of setae. Peritreme extends anteriorly to middle of coxa II. Coxae I, II, and III each with two slender setae. Coxa IV with a single slender seta. All coxae lack ventral spurs. Coxa II with a dorsal anterior spur. Tarsus II is like the female in lacking claw-like spines.

Dorsal plate reticulated, with about 20 pairs of short, fine setae and a much larger posterior pair of setae. The plate has at least 10 pairs of tiny pores; it is more narrowed posteriorly and covers much less of the dorsum than in the female. Uncovered dorsum striate, with about 16 pairs of setae.

Gnathosoma like the female except a pilus dentilus was not observed on the fixed chela.

Holotype. Female (ex nest of *Neotoma albigula albigula* Hartley, Mastin Ranch, location 25-C, Santa Fe County, New Mexico, 18 November 1952, H. B. Morlan, collector) deposited in the U.S. National Museum, No. 2126.

Allotype. Male (same collection data as holotype) deposited in the U.S. National Museum.

Paratypes. One each of the male and female (same collection data as holotype) to: Communicable Disease Center Museum, Atlanta, Georgia; Rocky Mountain Laboratory, Hamilton, Montana; Army Medical Service Graduate School, Washington, D. C.; R. W. Strandtmann, Lubbock, Texas; and H. B. Morlan, Santa Fe Field Station, Santa Fe, New Mexico. One each of the deutonymphs (ex nest of *Neotoma albigula albigula* Hartley, Mastin Ranch, location 75-A, Santa Fe County, New Mexico, 28 April 1953) to: U.S. National Museum, R. W. Strandtmann, and H. B. Morlan.

The new species has been found frequently in Santa Fe County, New Mexico, with *Hirstionyssus neotomae* in nests of the white-throated wood rat, *Neotoma albigula albigula*. Of 27 nests examined, both the new species and *H. neotomae* were present in 15 nests; only the new species was found in 3 nests; and only *H. neotomae* in 3 nests. Both species have been found during every month of the year. Deutonymphs of the new species were found in January, April, June, and July. Deutonymphs of *H. neotomae* were found in January, February, April, June, and September. Protonymphs have not been encountered.

Characters of the new species, by which it may be readily separated from the associated *H. neotomae*, include shape of sternal plate, lack of a ventral spur on coxa II, and the comparatively minute dorsal setae. The last two characters also serve to differentiate the males and deutonymphs.

Hirstionyssus occidentalis (Ewing), 1922. *Proc. U.S. Nat. Mus.*, 62: 20. Since Ewing illustrated only the anal plate of this mite, and his description is rather vague, we have included rather detailed drawings. Figures 32–35 are drawings made from the type. The body setae are rather weak and sparse. The two claw-like setae at the apex of tarsus II are practically straight rather than distinctly curved as in some other species possessing them. The tarsi of leg I and the tips of the chela are broken off.

Hirstionyssus santos-diasi Zumpt, 1951. *Moçambique*, 67:7. The description and illustration of this species require a few comments. He says, ". . . tibia (of leg II) of characteristic hook-like shape. . . ." The text figure makes it fairly certain that he meant tarsus II. It is because of this pronounced hook-like appearance that we assume *santos-diasi* to have the two claw-like setae at the apex of tarsus II, being similar in that respect to *creightoni* Hirst which has the claw-like structures.

Dr. Zumpt further states that there is a small tooth on the front margin of coxa I and coxa III. His text figure indicates that this is merely the hinge armature, or the point of junction between the coxa and trochanter. Although we cannot speak for *santos-diasi*, other species of *Hirstionyssus* do *not* lack the tritosternum.

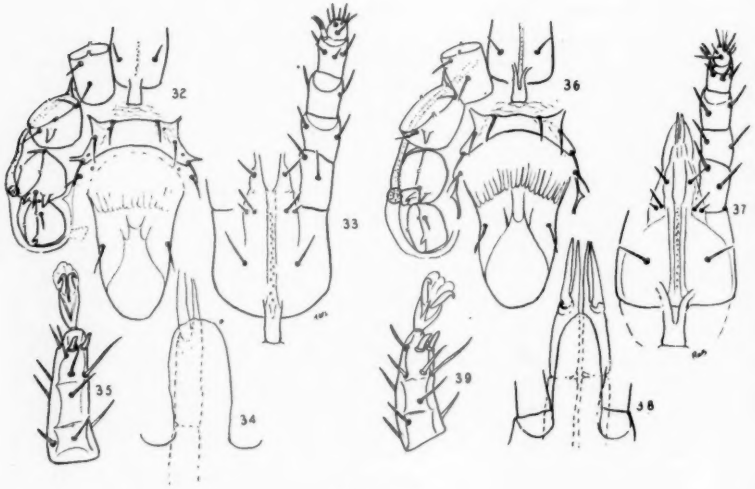

PLATE III

Female *Hirstionyssus occidentalis* (from U.S.N.M. type): Figure 32, ventral view; 33, gnathosoma; 34, tectum and chelicera; 35, tarsus II.

Female *Hirstionyssus cynomys* (from U.S.N.M. paratype): Figure 36, ventral view; 37, gnathosoma; 38, tectum and chelicera; 39, tarsus II.

Species known only from the male or nymph, incorrectly placed in *Hirstionyssus*, or too poorly described to classify:

Ichoronyssus albato-affinis Oudemans, 1902. A synonym of *Hirstionyssus carnifex* (C. L. Koch, 1839).

Ichoronyssus albatus (C. L. Koch), 1839. A synonym of *Hirstionyssus arcuatus* (C. L. Koch) 1836.

Liponyssus berlesei Hirst, 1921. A synonym of *Hirstionyssus confuscianus* (Hirst), 1921, the name *berlesei* having been preoccupied.

Lyponyssus chilensis Ewing, 1925. *Ent. News*, 36: 19. This species is sometimes listed as a *Hirstionyssus* but there is nothing in Ewing's original description to warrant this. In a note dated January 28, 1948, E. W. Baker, of the U.S. National Museum, says the type specimen is in poor shape, but that it looks like a typical Macronyssid of some sort.

Liponyssus cynomys Radford, 1941. *Parasit.*, 33: 313. A synonym of *Hirstionyssus occidentalis* (Ewing). The original drawings and description indicate that this species lacks a spur on coxa IV. However, a paratype female in the U.S. National Museum distinctly shows the presence of this character. The details of the paratype are shown in figures 36–39, and indicate it is a synonym of *occidentalis*. It will be noted that the peritreme is slightly longer and the sternal plate a trifle narrower, but otherwise there are no differences. Both *cynomys* and *occidentalis* were collected in Montana, U.S.A., although from different hosts.

Hirstionyssus hirsti Fonseca, 1948. *P. Zool. Soc.*, Lond., 118: 297. Synonym of *Liponyssus aethiopicus* Hirst, 1921, *in part*. Fonseca intended the name *hirsti* to apply only to the male of what Hirst called *aethiopicus;* the female is a bona fide *Bdellonyssus*. Apparently Fonseca meant Hirst's description of the male to suffice as he gives no description or illustration. It is placed in the genus *Hirstionyssus* on the following basis: "There is a rather strong, curved anterior spine on the anterior margin of the third coxa."

Hirstionyssus soricis (Turk), 1945. *Parasit.*, 36: 134. Only the male and deutonymph are known. From *Sorex minutus*, England. The setation is coarse, the dorsal setae are short and heavy except for the posterior pair on the dorsal plate, which are long and slender. The holoventral plate of the male has only three pairs of setae, all of which are posterior to Leg IV. The coxal spur pattern is 1–4–2–0. There is no mention of claw-like setae on tarsus two. The coxal spur pattern of the nymph is 0–3–0–0.

We extend our sincere appreciation to Edward W. Baker of the U.S. National Museum, Washington, D.C.; Charles D. Radford, Manchester, England; R. B. Eads, State Department of Health, Austin, Texas; and E. W. Jameson, Jr., the University of California, for the loan of type specimens. Also to E. O. Hunt, Bureau of Entomology and Plant Quarantine, for the loan of literature and for his interest and assistance earlier in this study.

BASAL METABOLIC CHANGES IN THE GUINEA PIG DUE TO SURGICAL REMOVAL OF AORTIC PARAGANGLIA*

OZRO B. WISWELL

Regarding elementary physiological changes, the fact must be emphasized that rate of oxygen utilization is an important index to the well-being of animals. While working on another phase of surgical removal of the aortic paraganglia of rats, and of guinea pigs (Baldwin *et al.*, 1943), it appeared that changes in metabolism (Wiswell, 1952) of these animals took place (Schmidt *et al.*, 1940). To determine the extent and fix, if possible, the quantitative limits of these physiological changes it was thought a series of oxygen utilization evaluations would prove interesting.

Zukerkandle (1901) has described the aortic bodies in the human as a pair of small elongated bodies located opposite the inferior mesenteric artery and in close proximity to the sympathetic aortic plexus. Piersol has stated that in 15 per cent of the cases the two bodies are joined by an isthmus into a horseshoe-shaped organ of varying dimensions, the right usually larger (right 11.6 mm., left 8.5 mm.). The extremes of length are 8 to 20 mm. on the right side and 3 to 15 mm. on the left side. The width is about one-fifth of the length and thickness considerably less. Gray, Morris and Shaffer, Cunningham and others have alluded to the aortic paraganglia as essentially an organ of fetal life or early childhood, and in the adult mere atrophic remains.

In the guinea pig the aortic paraganglia are located just posterior to the bifurcation of the dorsal aorta, and are in close proximity to the two common iliacs. Each aortic paraganglion in addition to having an excellent blood supply is closely related (at least morphologically) to the sympathetic nervous system. In a mature adult animal they are on the average about 10–12 mm. in length, 1–2 mm. wide and proportionately thinner. They are somewhat smaller in younger animals. The left aortic paraganglion is usually larger.

* From the Department of Anatomy, The University of Texas, Dental Branch, Houston. Presented before the annual meeting of the Federation of American Societies of Experimental Biology, New York, March, 1952. Received for publication July 13, 1953.

Materials and Methods. The aortic paraganglia were removed through the ventral wall under ether anesthesia. After removal of the aortic bodies the peritoneum and body wall were closed together by a simple continuous suture and the skin similarly brought together. The recoveries were without incident and the operations resulted in practically no mortality.

Sixty animals were used for the experiment, all approximately one year old. Tests were run pre-operatively on all animals to determine an average normal oxygen utilization rate. As the animals were fasted for 12–15 hours prior to each oxygen utilization determination this rate will hereafter be called the Basal Metabolic Rate (BMR), notwithstanding that CO_2 values were not determined. Twenty animals of each sex underwent the operative procedure, thus leaving ten of each sex to act as controls. The usual soda-lime CO_2 absorbent was used in the closed chamber-spirometer system. A small blower was included in the system.

Observations and Discussion. Considering the male animals first as a group, the post-operative results were as follows: there was a continued average increase during the first 15 post-operative days when the BMR reached a peak of 15 per cent above the average pre-operative level. On the 28th day the average BMR for the group had returned to the normal basal level but continued to show a steady decline, until the experiment was terminated on the 90th post-operative day when the BMR was 19 per cent below the pre-operative average level. In the control group of males, the BMR over the 90 day interval fluctuated only slightly.

The BMR of the female animals varied somewhat from that of the males. The highest post-operative level was on the 10th day when the BMR rose 2.5 per cent above the pre-operative normal level for the sex, but declined rapidly so that by the 16th day it was back to the pre-operative level. This group of females also underwent a continuous decline in BMR but apparently in proportion, not to such a marked degree as in the males. On the 40th day the BMR of this group started to level off and on the 90th day of the experiment had dropped 13 per cent below the average BMR normal (Fig. I).

As a check against the validity of the foregoing data several control animals were subjected to mock operations; in these the aortic paraganglia were not removed. They showed as a result only a post-operative increase for several days then returned to the pre-operative normal.

Conclusions. These data, therefore, indicate that: 1. There is

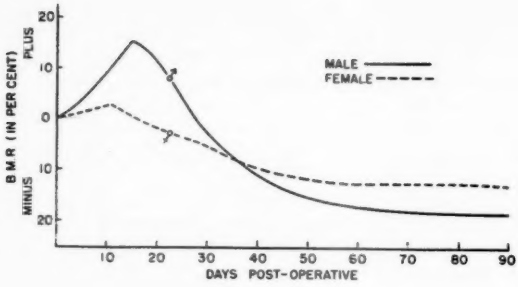

FIGURE I.

an interesting series of post-operative changes in the oxygen utilization rate during a certain post-operative period in both male and female groups. 2. Animals subjected to this operative procedure decreased in their oxygen utilization in relation to their body weight. 3. Because of this reduction in the oxygen utilization rate it would seem to indicate that these aortic paraganglia have physiological significance.

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M. D. ANDERSON HOSPITAL FOR CANCER RESEARCH, SEVENTH ANNUAL SYMPOSIUM, 1953

R. Lee Clark, Jr.

Introduction

It has been said that the key to the cancer problem must eventually be found at the *fundamental* research levels of biology, biochemistry, pathology, and related fields. Giving full credence to this statement, The University of Texas M. D. Anderson Hospital for Cancer Research instituted an Annual Symposium on Fundamental Cancer Research in 1946. This action was taken because of the institution's dedicated interest in basic research. We have invited as speakers at these meetings some of the most outstanding investigators in fundamental research, hoping thereby to establish a gathering wherein important current research could be reported, discussed, and evaluated. This year, looking back over the seven symposiums that have been held, we feel justified in believing that the symposium idea is accomplishing its desired goal.

The Seventh Annual Symposium, held May, 1953, was attended by persons from 16 states and four foreign countries. Again this year, following the plan adopted in the more recent symposiums, a part of the program was constructed around a single theme—*Newer Methods of Microscopy and Cell Research.* Theme subjects at earlier symposiums have included: isotopes in cancer research, protein metabolism and relation to cancer, and nutritional factors in cancer research. The current subject is particularly "fundamental" and, we believe, particularly important to the study of neoplasia. Dr. Robert C. Mellors summarized the significance of this year's theme in his opening remarks at the meeting: "Since the origination of the cell theory, its establishment in biology by Schleiden and Schwann, and its application to pathology by Virchow, the microscope has played an important and historic role in biology and medicine. The microscope has now been developed to utilize all of the properties of a light wave—the amplitude, the frequency, and the phase and the plane of vibration—and to employ in addition to electromagnetic radiations other forms, or carriers, of radiant energy,

such as an electron beam. It is the purpose of this Symposium to describe some of the recent advances in methods of microscopy of the cell, to present the results obtained, and to indicate their manifest or potential significance in cancer research."

In addition to the papers on the theme subject, certain other reports were presented and are published in the symposium issue. These include papers on cancer given at the Joint Meeting of the Symposium with the South Central Region College of American Pathologists, the Texas Society of Pathologists, and the Houston Society of Pathologists.

Of course, the high point of the annual Symposium is the presentation of the Bertner Foundation Award and the deliverance of the Bertner Lecture. The Bertner Award was established in 1950 in honor of the late Dr. E. W. Bertner, first acting director of the M. D. Anderson Hospital and first president of the Texas Medical Center. Presented in the form of a medallion, this award is given to a person who the Awards Committee feels has made an outstanding contribution to cancer research during the year. Recipient of this year's award was Dr. Charles B. Huggins of the University of Chicago, who also presented the Bertner Lecture.

Each year, the Symposium papers have been published in *Texas Reports on Biology and Medicine*, through the courtesy and co-operation of Dr. Chauncey D. Leake, Director of The University of Texas Medical Branch and editor of this journal. We express our sincere gratitude to Dr. Leake and his staff for their contributions which help to make the Annual Symposiums have lasting value.

HISTOCHEMICAL LOCALIZATION OF SUCCINIC DEHYDROGENASE AND ENDOGENOUS REDUCTASE ACTIVITY IN SQUAMOUS CELL CARCINOMA OF THE CERVIX*

ALVAN G. FORAKER† AND SAM W. DENHAM‡

Succinic dehydrogenase plays a vital role in respiratory processes of most living cells and forms a link in the chain reactions concerned with the oxidation of lipids, carbohydrates and proteins (Seligman and Rutenburg, 1951). In recent years, histochemical methods for the demonstration of succinic dehydrogenase have provided means for determining the sites of activity of this oxidative enzyme within cells and tissues (Padykula, 1952). The principle of the demonstration is the observation of the change in color of a suitable hydrogen acceptor (one of the various tetrazolium salts) when it is reduced by the enzyme in the presence of a suitable substrate (Gomori, 1952).

Previously we found evidence of dehydrogenase activity in cervical biopsies from pregnant and nonpregnant women corresponding to regions of cell growth, as in the basal layer of portio vaginalis squamous epithelium (Foraker and Denham, 1953).

In the present series of cervical biopsies from cases of squamous cell carcinoma, succinic dehydrogenase activity and endogenous reductase activity were demonstrated within the cytoplasm of the neoplastic cells.

Materials and Methods. The histochemical technique was that of exposing the tissue to neotetrazolium (NT) (pp′ diphenylene bis 2-(3,5 diphenyl tetrazolium chloride)) (Antopol, Glaubach and Goldman, 1950) in the presence of an excess of succinate. Neotetrazolium is pale yellow in solution and is converted to an insoluble deep purple to black compound of the formazan group at the site of active metabolic processes.

* From the Departments of Pathology, and Obstetrics and Gynecology, Emory University School of Medicine and Grady Memorial Hospital, Atlanta, Georgia. Presented at the Seventh Annual Cancer Symposium, May, 1953, at the M. D. Anderson Hospital for Cancer Research, Houston. This investigation was supported by a research grant from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

† Present address: M. D. Anderson Hospital for Cancer Research, Houston, Texas.

‡ Present address: St. Vincent's Hospital, Jacksonville, Florida.

The technique was modified from that reported by Rutenburg, Wolman, and Seligman (1953). The modifications were planned to meet the exigencies of collecting human material in clinics.

The tissue was obtained from twelve women with squamous cell carcinoma previously diagnosed by biopsy, but who had received no radiation therapy. Biopsies of cervices of other women were obtained for comparison.

The following phenomena are of importance in planning this technique and in evaluating the results of the study:

1. Even without the use of any substrate, positive reactions will be obtained in most cases on account of the presence of various endogenous substrates in the tissues (Gomori, 1952). Hence incubation of the tissue with NT under suitable circumstances will result in reduction of some of the tetrazolium salt and deposition of formazan pigment (Antopol, Glaubach, and Goldman, 1950). This undefinable substrate activity must be removed before acceptance of the reaction as valid for succinic dehydrogenase demonstration.

2. Freezing the tissue effectively destroys this endogenous activity (Shelton and Schneider, 1952).

3. When the endogenous activity has been thus destroyed, as demonstrated by preparation of control material, tissue incubated in NT with an excess of succinate should demonstrate sites of succinic dehydrogenase activity (Shelton and Schneider, 1952).

Fresh biopsies of cervical tissue obtained in the clinic were trimmed to blocks not exceeding three millimeters in thickness and processed as follows:

A. One or more blocks from each case were frozen on the freezing microtome for a period of 15 minutes to destroy endogenous activity. These blocks were then incubated for one hour at 37° C. with gentle agitation in 8.0 cc. of 1.2 per cent NT in 0.1 M. phosphate in normal saline buffered to pH 7.4 with the addition of 2.0 cc. of 0.3 M. sodium succinate. This preparation was designed to show specific succinic dehydrogenase localization.

B. One or more blocks from each case were frozen on the freezing microtome in parallel with the preceding (A) tissues. Incubation in the NT solution with no added succinate followed. This preparation was designed as a negative control to prove the destruction of endogenous substrate and reductase activity.

C. One or more blocks from each case were placed directly in the NT solution with added succinate, with no preliminary freezing. Incubation followed as above. This preparation was designed to show endogenous tissue activity as well as any enhancement of the reaction induced by the addition of an excess of succinate.

D. One or more blocks from each case were placed directly in the NT solution without added succinate, with no preliminary freezing, then incubated as above. This preparation was designed to show endogenous tissue activity only.

All blocks were then fixed in neutralized formalin. Frozen sections (15 μ) from these blocks were mounted in gum arabic. The blocks were then de-

hydrated in dioxane (which removed all of the formazan), embedded in paraffin and sections (5 μ) were cut. These were stained with hematoxylin and phloxine.

Results

After incubation, gross examination of the blocks in general showed the following changes:

1. Moderate reddish or light blue tinging of the blocks frozen then incubated in NT with succinate (SDH only).
2. Little or no color change in the blocks frozen then incubated in NT without succinate (control).
3. Dark reddish purple color of the cut surfaces of blocks placed fresh in NT with succinate (SDH plus endogenous) and in NT without succinate (endogenous only).

In the frozen sections reduced NT was seen in two forms, as black or dark brown pigment granules and as reddish microdroplets. The deposition of the granules was confined to the cytoplasm, with little or no reaction discernable in nuclei. The red microdroplets occurred largely in the cytoplasm, but some appeared to be extracellular. In both non-neoplastic and neoplastic epithelium the pattern of distribution in all three types of test sections (SDH only, SDH plus endogenous, and endogenous only) was essentially the same, varying in degree as noted below.

In the normal portio vaginalis epithelium (Fig. I), formazan deposition was largely confined to the basal layer, with lesser deposition in immediately adjacent epithelial layers. In the sections showing both SDH and endogenous reaction (Fig. I-C) the vessels in the stroma were lightly outlined by formazan granules.

In metaplastic squamous epithelium (Fig. II) the reaction was again maximal in the basal layer, but was seen more diffusely through superficial layers.

In regions of intraepithelial cervical carcinoma (Fig. III), formazan deposition was seen diffusely through the epithelial layer but was somewhat more intense in the deeper portions.

In areas of invasive squamous cell carcinoma (Fig. IV), the reaction was seen rather diffusely through the neoplastic cells. However, the formazan deposition was more intense at the outer

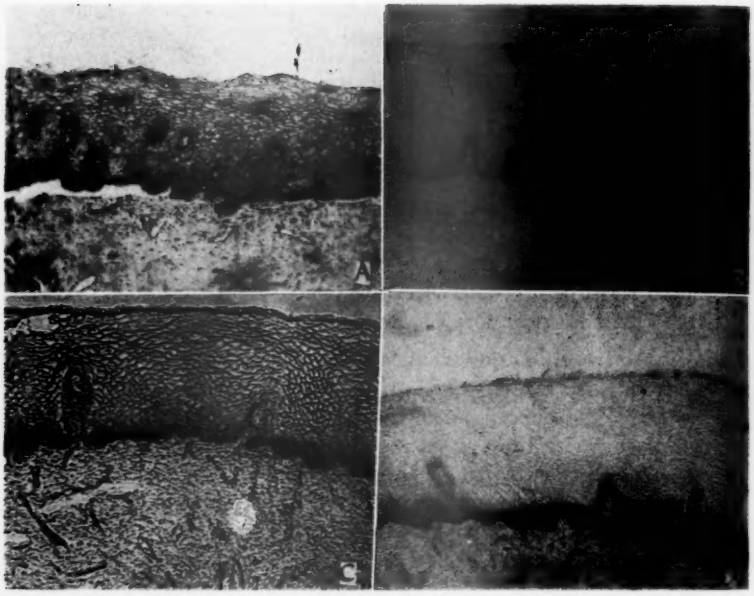

FIG. I. Normally maturing squamous epithelium of portio vaginalis of cervix (X 75).

A. Hematoxylin and phloxine section.

B. SDH only. Frozen section of same tissue incubated in NT with succinate to demonstrate sites of succinic dehydrogenase activity. Dark granules of reduced NT largely confined to basal layer of epithelium.

C. SDH plus endogenous reductase. Frozen section of same case incubated fresh in NT with succinate to demonstrate sites of dehydrogenase activity (endogenous reductase with enhancement of effect of succinic dehydrogenase). Same distribution of formazan granules as in B, but reaction considerably heavier. Vessels in stroma lightly outlined by NT granules.

D. Endogenous reductase only. Frozen section of same case incubated fresh in NT without succinate to demonstrate sites of endogenous reductase activity. Much the same distribution pattern as C.

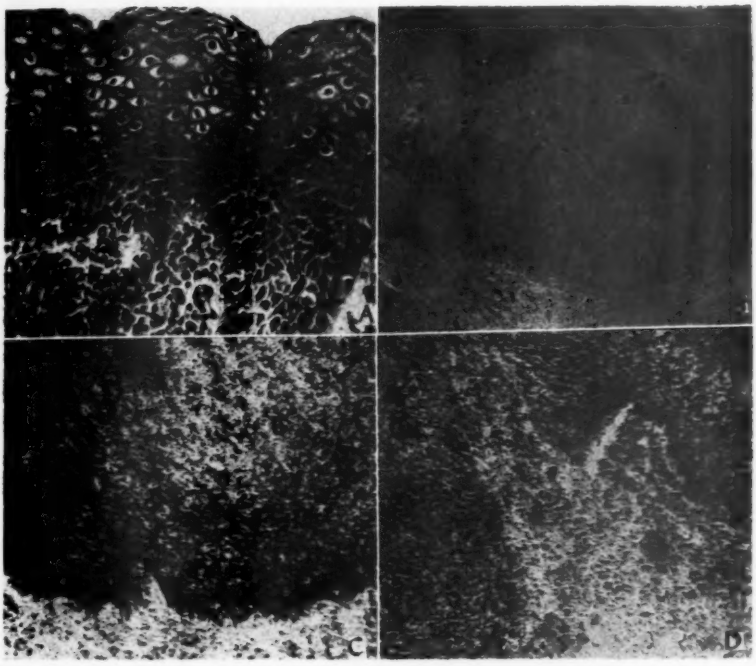

FIG. II. Metaplastic squamous epithelium overlying endocervical tissue (X 330).

A. Hematoxylin and phloxine section.

B. SDH only. Extremely light reaction consisting in large part of red microdroplets occurring through epithelial layer.

C. SDH plus endogenous reductase. Diffuse deposition of formazan granules and red microdroplets in basal layer and somewhat lighter deposition in overlying layers.

D. Endogenous reductase. Much the same distribution of formazan as in C, but reaction considerably lighter.

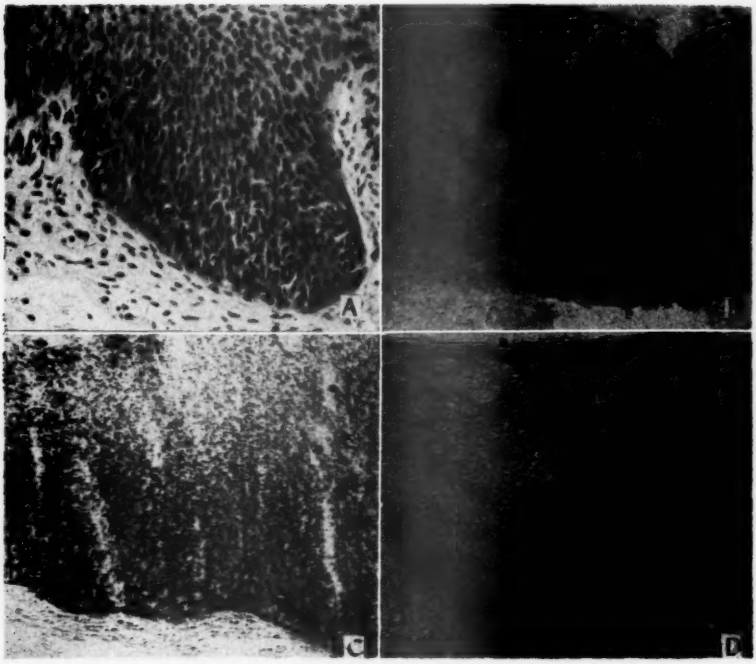

FIG. III. Intraepithelial carcinoma of cervix (X 330).

A. Hematoxylin and phloxine section showing lack of maturation and other features of intraepithelial carcinoma.

B. SDH only. Rather light reaction of red microdroplets and blue formazan granules extending through epithelial layer.

C. SDH plus endogenous reductase. Heavy reaction of blue formazan granules extending through epithelial layer. Notice clear areas in centers of cells corresponding to location of nuclei.

D. Endogenous reductase. Much the same distribution pattern of formazan granules as in C but reaction somewhat lighter.

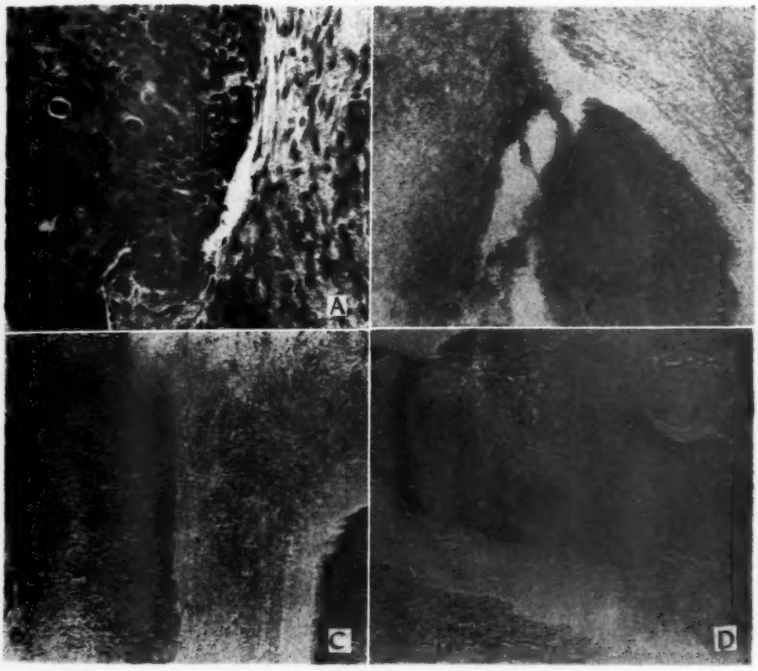

FIG. IV. Invasive squamous cell carcinoma of cervix (X 330).

A. Hematoxylin and phloxine section.

B. SDH only. Reaction of red microdroplets and blue formazan granules occurring diffusely through neoplastic cells.

C. SDH plus endogenous reductase. Reaction of blue formazan granules in neoplastic cells, but more prominent in cells at periphery of invasive strands. Note clear areas in centers of cells corresponding to locations of nuclei.

D. Endogenous reductase. Much the same as C, but reaction somewhat lighter. Epithelial pearl in upper right corner contains no formazan granules.

cell layers of invasive strands of carcinoma. Pearls of keratinizing epithelium (Fig. IV D) showed little or no reaction.

In all cases the control sections (frozen then incubated in NT without succinate) showed no reaction or very rare presence of a reddish microdroplet.

The sections designed to show SDH activity only (frozen then incubated in NT with succinate) usually revealed rather light reaction, being less than half as strong as the types of section discussed in the next two paragraphs.

The sections designed to indicate endogenous tissue activity, with enhancement of the effect of succinic dehydrogenase (fresh block incubated in NT with succinate) contained definite clear-cut reaction, distributed as noted above.

The sections planned to show endogenous reductase activity only (fresh block incubated in NT without succinate) showed definite clearcut reaction, about one half to two thirds as strong as blocks incubated in NT with succinate.

Discussion. It is felt that little would be gained from considering the comparative intensity of the formazan deposition in the three variants of the technique. It is doubtful whether the formazan deposition in the sections designed to reveal specific succinic dehydrogenase activity is present in a degree proportional to the actual fraction of total reductase activity furnished by this enzyme. The freezing employed to destroy endogenous activity decreases the enzymatic activity to a significant extent in some tissues (Goddard and Seligman, 1952). In addition, certain techniques likely to enhance the reaction such as partial anaerobiasis, or addition of certain metallic ions (Rutenburg, Wolman, and Seligman, 1953) were not employed in this study. The reactions were somewhat patchy within each section, further vitiating any prospect of deriving quantitative data concerning the degree of enzyme activity from the degree of the staining reaction.

The finding of the reaction virtually confined to cytoplasm with little or no nuclear formazan deposition is consistent with observations by workers studying various human and animal tissues (Foraker and Denham, 1953; Padykula, 1952; and Seligman and Rutenburg, 1951). The significance of the finding of red microdroplets in certain areas rather than blue formazan

granules must be inferred. The diformazan is soluble in lipid and appears red by transmitted light in lipid solution (Rutenburg, Wolman, and Seligman, 1953). The possibility must also be considered that part of the red staining could have been due to incomplete reduction of the tetrazolium salt to a red monoformazan. In lipid poor cells, such as the squamous epithelial cells of the current study, this latter assumption seems more likely.

Succinic dehydrogenase has been shown to be firmly attached within the cell quite probably to mitochondria (Padykula, 1952). The enzyme is morphologically coupled with mitochondria (Goddard and Seligman, 1952). However, our present study permits of no observation in this respect since intracellular organelles cannot be distinguished with frozen sections (Goddard and Seligman, 1953).

The patterns of formazan deposition indicating specific SDH, endogenous reductase and combined reaction were essentially the same in distribution. In normal portio vaginalis squamous epithelium they indicated maximum activity in or near the basal layer, where cell proliferation is most apparent. In metaplastic, noninvasive and invasive neoplastic squamous epithelium, they were more diffusely distributed through the epithelial cells. These findings are consistent with the assumption that actual cell multiplication and proliferation occurs most actively at the basal layer of the normally maturing portio vaginalis epithelium, but are more diffuse in the abnormal epithelium of all three types (metaplastic, noninvasive and invasive neoplastic).

Summary. Sites of succinic dehydrogenase and endogenous reductase activity were studied in invasive squamous cell carcinoma, intraepithelial carcinoma, metaplastic and normal portio vaginalis squamous epithelium of the cervix uteri by incubating tissue blocks in neotetrazolium. In general manifestation of enzyme activity occurred in the basal layer of normally maturing squamous epithelium but more diffusely through the other types of epithelium. This corresponds in general to sites of cell proliferation.

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THE IMPORTANCE OF SULFHYDRYL GROUPS IN BIOLOGY AND MEDICINE

E. S. Guzman Barron*

Sixty-five years have elapsed since deRey-Pailhade discovered the presence of "philothion" in living cells, and thirty-two since Hopkins rediscovered it and named it glutathione. The investigations which have been performed during this time have greatly advanced our knowledge, and the role of the sulfhydryl compounds is becoming clear. The —SH groups present in proteins have been extensively studied, and their importance for the activity of enzymes has been recognized; moreover, the mechanism of action of the —SH groups in enzymes and other proteins is beginning to be understood. In the same manner, the role of glutathione as a regulator of cellular metabolism and as a coenzyme has also been clarified. Lately —SH and —SS coenzymes—Coenzyme A, lipoic acid—have been discovered, thus broadening the biological importance of these compounds. The role of —SH groups in cellular growth and cell division, discovered twenty years ago by Rapkine, Hammett, and Voegtlin, still remains to be elucidated. The great reactivity of the —SH groups has made them vulnerable to the action of many agents; as a consequence, destruction of these groups has been found to be the base for a variety of pathologic disorders, such as heavy metal intoxications and ionizing radiation diseases.

—*SH Reagents.* Perhaps the most important reason for the progress made in the field of sulfhydryl compounds in biology is the reactivity of the —SH groups: they are in general easily oxidized, they form with heavy metals reversible mercaptides, and they are easily alkylated (Table 1). The use of these three kinds of reagents has facilitated the discovery of —SH groups in substances of biological importance.

—*SH Proteins.* The presence of —SH groups was discovered forty-six years ago by Haffter, who found that denatured, but not native, egg albumin gave a strong nitroprusside test. It is now

* From the Chemical Division, the Department of Medicine, the University of Chicago. Presented at the Seventh Annual Cancer Symposium, May, 1953, at the M. D. Anderson Hospital for Cancer Research, Houston.

known that some proteins (myosin, certain enzymes) have freely reacting —SH groups in the native state, whereas others possess sluggish —SH groups, *i.e.*, groups that are not easily attacked or that react with —SH reagents only after denaturation (egg albumin, serum albumin).

TABLE 1

Sulfhydryl Reagents of Biological Importance

Reaction	*Reagents*
Oxidation	Ferricyanide, Iodosobenzoic acid Hydrogen peroxide, Porphyrindine Alloxan, Cupric salts 2,6-dichlorophenolindophenol
Alkylation	Iodoacetate, Bromoacetate Iodoacetamide, chloropycrin Sulfur mustards, Nitrogen mustards
Mercaptide-formation	p-chloromercuribenzoic acid, Salirgan (sodium salt of O-C (3-hydroxymercuri-2,-methoxypropyl) carbonyl) phenoxyacetic acid, trivalent organic arsenicals, cadmium chloride.

The protein moiety of a large number of enzymes—the —SH enzymes—requires the presence of —SH groups for enzymatic activity. Some of these enzymes (adenosinetriphosphatase, alcohol dehydrogenase) seem to require all their —SH groups, for loss of enzyme activity is proportional to the addition of sulfhydryl reagent. Others (urease) require only a portion of their —SH groups for activity. The first group of enzymes found to need the presence of —SH groups for activity were the hydrolytic enzymes (Table 2). Later investigations have extended consid-

TABLE 2

Hydrolytic Enzymes Requiring —SH Groups

β-Amylase	Esterase (pancreatic)
Asclepain m (milkweed)	Fixin
Asclepain s (milkweed)	Hemolysin (Pneumococcus)
Bromelin (pineapple)	
Carboxylase	Lipase
Cathepsins	Hemolysin (cobra venom)
Cerebroside	Papain
Choline esterase	Urease
Carboxypeptidase (pancreatic)	Miscellaneous: Conjugase Transaminase

erably their number. A large number of oxidation-reduction enzymes (Table 3) and possibly all enzymes dealing with adenosi-

TABLE 3

Oxidation-Reduction Enzymes Requiring —SH Groups

Enzyme	Oxidation-reduction system
Alcohol dehydrogenase (yeast)	DPN
Acetaldehyde dehydrogenase (yeast)	DPN
β-Hydroxybutyric dehydrogenase	DPN
Glutamic dehydrogenase	DPN
α-Ketoglutaric acid oxidase	DPN
Malate dehydrogenase	DPN
Glycerol oxidase (bacteria)	DPN
Phosphoglyceraldehyde dehydrogenase	DPN
Betaine aldehyde dehydrogenase	DPN
Pyruvate oxidase	DPN
Pyruvate dismutation	DPN
α-Amino acid oxidase	Flavin
Xanthine oxidase?	Flavin
Choline oxidase	Flavin, Cyto.*c*
Succinic dehydrogenase	Flavin, Cyto.*c*
Stearate dehydrogenase	DPN
Oleate dehydrogenase	DPN
Monoamine dehydrogenase	?

netriphosphate transfer reactions (Table 4) have been shown to be —SH enzymes. The list has not yet been completed. As a matter of fact, we have found recently (unpublished observations of H. Rothschild and Barron) that betaine aldehyde dehydrogenase is an —SH enzyme.

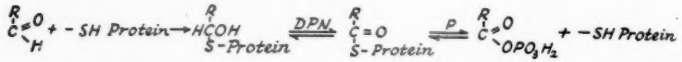
TABLE 4

ATP-Enzymes Requiring —SH Groups

Enzyme	Process
Phosphoglucomutase	P-transfer
Myokinase	P-transfer
Adenosinetriphosphatase	P-hydrolysis
Phosphorylase (potato)	P-transfer
Hexokinases	P-transfer
Creatine phosphokinase	P-transfer
Phosphopyruvate phosphokinase	P-transfer

The reason for the necessity of —SH groups is not yet known with certainty. Theorell and Bonichsen believe that coenzymes anchor on the —SH groups (reduced DPN attached to the —SH

groups of alcohol dehydrogenase). Racker and Krimsky have postulated that phorphoglyceraldehyde combine with the —SH group of the protein and is then phosphorolytically split with the formation of 1,3-phosphoglyceric acid:



It is well known that aldehydes combine reversibly with —SH groups (Levi, 1932; Ratner, 1937). It is also possible that the —SH groups of the protein in the form of a free-radical act as electron mediators between substrate already combined to the protein and the oxidation-reduction catalyst (Barron, 1952):

$$CH_3CH_2OH + \dot{S}\text{-Protein} \rightleftharpoons CH_3\dot{C}HOH + {}^-\text{SH Protein} \quad (1)$$

$${}^-\text{SH Protein} + DPN^+ \rightleftharpoons \dot{S}\text{-Protein} + DP\dot{N}H \quad (2)$$

$$CH_3\dot{C}HOH + \dot{S}\text{-Protein} \rightleftharpoons CH_3COH + {}^-\text{SH Protein} \quad (3)$$

$${}^-\text{SH Protein} + DP\dot{N}H \rightleftharpoons \dot{S}\text{-Protein} + DPNH + H^+ \quad (4)$$

Biological oxidations proceed by univalent steps with the intermediate formation of free-radicals, according to the postulates of Michaelis.

Myosin has —SH groups which play an important role in the process of muscular contraction. For its enzymatic activity it requires —SH groups (Singer and Barron, 1944). Moreover, the combination of actin with myosin seems to take place through the —SH groups of myosin (Bailey and Perry, 1947). Since adenosinetriphosphatase activity and actomyosin formation are essential for muscle contraction, it must be concluded that —SH groups are necessary for this physiologic process.

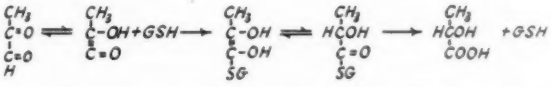
The influence of —SH groups in another physiologic process—blood coagulation—was suggested by the observation of Kühnau and Morgenstern that glutathione and cysteine inhibited the coagulation of fresh guinea pig and human blood whereas oxidized glutathione accelerated it. It seems that coagulation starts with the hydrolysis of fibrinogen by thrombin A, which converts the sluggish —SH groups of the molecule into freely reacting —SH groups (a process similar to that of the denaturation); and that these fibrinogen molecules attach themselves to each other through their —SH groups under the action of thrombin B

(Lyons, 1945; Jaener, 1947). Fibrin would thus be a disulfide fibrinogen.

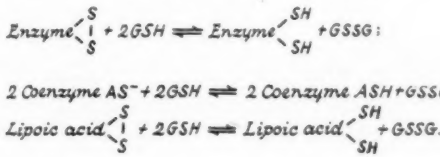
Glutathione. Of all the substances of biological importance, glutathione seems to be the most universally distributed, as it is present in animals and plants; in aerobic and anaerobic bacteria. A sluggish oxidation-reduction system, it is easily oxidized by the catalytic action of Cu^{++} and Fe^{+++}. Its biological synthesis, as shown by the studies of Bloch (Yanari and Bloch, 1953; Snoke and Bloch, 1953), is performed by two enzymes, of which one conjugates glutamic acid with cysteine, and the other conjugates glutamylcysteine with glycine:

L-glutamic acid + L-cysteine + ATP → L-γ-glutamyl-L-cysteine.

L-γ-glutamyl-L-cysteine + glycine + ATP → Glutathione. It seems that the energy necessary for the peptide bond formation is provided by ATP, as one mole of inorganic phosphate was liberated per mole of glutathione synthesized. Glutathione acts as a coenzyme in the formation of lactic acid from methylglyoxal, a process whose mechanism has been elucidated by Racker. Two separate enzymes are required: one for the condensation between methylglyoxal and glutathione, and the other for the breakdown of this condensation product into glutathione and lactic acid:



The —SH groups, being easily oxidized, are continuously transformed into disulfide by the numerous oxidizing agents present in biological fluids. Glutathione, as a powerful reducing agent, reduces these inactive disulfides into the active —SH groups; the —SH enzymes, oxidized Coenzyme A; and lipoic acid can thus be reactivated by glutathione:

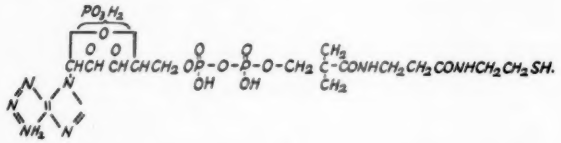


Glutathione keeps the —SH enzymes and the —SH coenzymes in the reduced, active form. The oxidized glutathione is then reduced by reduced triphosphopyridine nucleotide and the enzyme glutathione reductase present in animal and vegetable tissues (Rall and Lehninger, 1952; Conn and Vennesland, 1951):

$$GSSG + TPNH + H^+ \rightleftarrows 2GSH + TPN^+.$$

Glutathione can be considered the most universally present regulatory mechanism of cellular metabolism, as has been shown in experiments with sea urchin sperm (Barron, Nelson, and Ardao, 1948). —SH reagents, when used in small concentrations, increased the respiration of these cells, whereas the respiration was inhibited with increased concentrations. The increased respiration may be attributed to withdrawal of glutathione by combination with the —SH reagent and elimination of its regulatory power; the inhibition is due to destruction of the —SH enzymes.

Coenzyme A. Elucidation of the mechanism of action of coenzyme A and of its composition has greatly increased the importance of the —SH groups. According to Lipmann, who has contributed greatly to our knowledge of this coenzyme, it seems to have the following structure:



The role of the —SH group was discovered by Lynen who demonstrated that the "active acetate" postulated to be formed on oxidation of pyruvate or on the breakdown of citric acid (Lipton and Barron, 1946), is an acetyl-mercaptide of the type:

$$R\text{-}S\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_3.$$

Like other —SH compounds, coenzyme A was found to combine with iodoacetic acid to form the alkylated derivative:

$$CoASH + CH_2ICOO^- \rightarrow CoASCH_2COO^- + HI.$$

In acyl-mercaptides the S bond has the chemical properties of an acid anhydride and is, like ATP, a high energy bond. There is liberation of 12,000 calories in acetyl CoA (Stern, Ochoa and Lynen, 1952). The SH group, as a derivative of H_2S partakes the properties of a weak acid, and, as Lynen maintains, the high-energy S-bond is more stable, under pH and temperature conditions existing within physiologic limits, than the high-energy P-bonds. In all the reactions where there is acyl-coenzyme A formation there is liberation of high energy in the same manner as in the phosphorylated compounds. How much of the free-energy utilized by the cell is provided by these thioclastic reactions remains to be determined.

The great reactivity of acyl-coenzyme A compounds is being established by the combined efforts of the laboratories of Lipmann, Ochoa, Lynen, and Green. The numerous reactions in which coenzyme A acts as a catalyst may be classified in five, each of which is illustrated in table 5.

TABLE 5

Sulfhydryl Groups as Coenzymes: Coenzyme A (CoA-SH)

I. *Acyl Formation:*

Acetate + CoA-SH + ATP $\leftrightarrows$ Acetyl-S-CoA + AMP + pyrophosphate

Butyrate + CoASH + ATP $\rightleftarrows$ Butyryl-S-CoA + AMP + pyrophosphate

II. *Transacetylation:*

Acetyl PO_4 + CoASH $\leftrightarrows$ Acetyl-S-CoA + Phosphate

Acetylcholine + CoASH $\rightleftarrows$ Acetyl-S-CoA + Choline

III. *Transfer:*

Acetyl-S-CoA + Propionate $\leftrightarrows$ Propionyl-S-CoA + Acetate

Succinyl-S-CoA + Acetoacetate $\rightleftarrows$ Acetoacetyl-S-CoA + Succinate

IV. *Oxidation:*

Butyryl-S-CoA + DPN$^+$ $\rightleftarrows$ Crotonyl-S-CoA + DPNH + H$^+$

Crotonyl-S-CoA + H_2O $\rightleftarrows$ β OH butyryl-S-CoA

β-OH butyryl-S-CoA $\leftrightarrows$ + DPN$^+$ $\rightleftarrows$ Acetoacetyl-S-CoA + DPNH + H$^+$

V. *Condensation:*

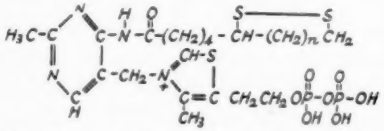
2 Acetyl-S-CoA $\rightleftarrows$ Acetoacetyl-S-CoA + CoASH

2 Acetyl-S-CoA + DPN$^+$ + H_2O $\leftrightarrows$ Succinate + 2 CoASH + DPNH + H$^+$

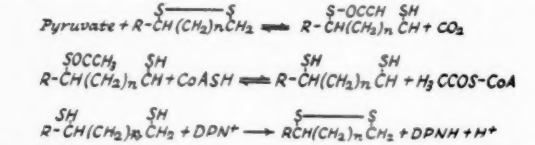
Acetyl-S-CoA + Oxalacetate $\rightleftarrows$ Citrate + CoASH

Benzoyl-S-CoA + Glycine $\leftrightarrows$ Hypurate + CoASH

Lipoic Acid Conjugate. ∝ Lipoic acid conjugate, the "protogen" or acetate-replacing growth factor of G. W. Kidder, and the pyruvic acid oxidase-factor of Gunsalus, seems to be formed by the combination of diphosphothiamine with ∝-lipoic acid (Reed and De Busk, 1952).

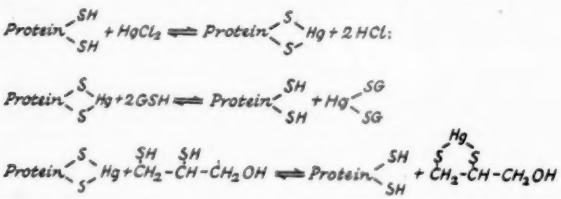


This conjugate is essential for the oxidation of pyruvic and ∝-ketoglutaric acids. According to Reed oxidation of these acids takes place as follows:



For the entrance of pyruvate into the tricarboxylic acid cycle, and for the continuation of this cycle (oxidation of ∝-ketoglutaric acid) there are required two S-containing coenzymes; the disulfide lipothiamide, and the sulfhydryl coenzyme A. The amount of these coenzymes will thus determine the contribution of the citric acid cycle to cell metabolism.

Heavy Metal Intoxications. It has been mentioned that —SH groups react reversibly with heavy metals (Hg, Bi, Cd, V, Pb, Sb, Xn, Ag), forming mercaptides. Trivalent arsenic and tellurium also combine reversibly, forming thioarsenites and thiotellurites. The toxic action of all these metals is due to combination with the —SH groups of biological importance. The mercaptides that are formed may be split on addition of sulfhydryl compounds, such as glutathione, cysteine, or dithiols:



Inhibitor and reversing agent compete for the —SH groups of the protein. If the competitor has greater affinity for the inhibitor than the protein has or is used in large concentrations, re-formation of the —SH groups of the protein will result. According to Peters, the dithiols are the best reversing agents, because they form the more stable cyclic compounds. The success of BAL (2,3-dimercaptopropanol) therapy is a demonstration of the correctness of Peters' contention. There is great variability in the reversing power of thiols: whereas some enzymes can be completely reversed, others are only partly reversed, and there are a few that cannot be reversed at all. There is also great varia-

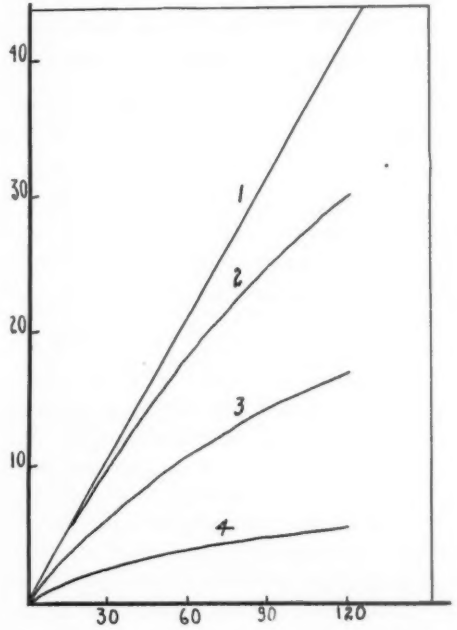


FIG. I. Effect of arsenicals on the respiration of kidney slices (rat) in the presence of glucose. Ringer-phosphate, pH 7.4. Temp. 38°. Abscissa, time in minutes; ordinate, Oxygen uptake in cmm. per mg. dry weight. 1. control; 2. Na arsenite; 3. p-carboxyphenylarsine oxide; 4. lewisite (chlorovinyl dichloroarsine) concentration of arsenicals 1×10^{-4} *M*.

tion in the inhibiting effect of the same mercaptide-forming agent, as can be seen in the effect of arsenicals on the respiration of kidney slices (Fig. I). In general, at equal —SH concentrations, BAL is the most powerful reactivating agent; glutathione comes next; and finally cysteine. A comparative study of the reversing power of BAL and six other dithiols against inhibition of succinic dehydrogenase by heavy metals showed that while BAL could reverse inhibitions produced by Hg, Bi, Cd, V, Zn, Pb, and Bi, other dithiols could reverse them at lower conecntrations than those required by BAL. Glutathione, at equal concentrations, was generally ineffective (Table 6).

TABLE 6

Comparison of Toxicity of Heavy Metals for Succinoxidase and Best Reversal Agents

Inhibitor	Half-inhibition	90% Inhibition	Best reversal agent
	M	*M*	
Bismuth	1.2×10^{-5}	3.0×10^{-5}	$2 > 1 > 4 = 3$
Mercury	1.25×10^{-5}	3.6×10^{-5}	$2 > 1, 4 = 3$
Cadmium	7×10^{-6}	1.3×10^{-5}	$2 = 1, 4 = 3$
Vanadium	1.1×10^{-4}	2.1×10^{-4}	2; 3 ineffective
Selenium	1.1×10^{-4}	4.0×10^{-4}	$1 = 2$
Zinc		2×10^{-3}	

1 = 1,3-dithiopropanol; 2 = propanedithiol 1, 3; 3 = 2,3-dithiopropanol (BAL); 4 = 2,3-dithiopropionic acid.

Sulfhydryl Groups and Hormones. Alloxan is one of the well-known —SH reagents (Labes and Freisberger, 1930). The production of diabetes in animals after the injection of alloxan; its prevention by previous injection of thiols, such as glutathione, cysteine, and BAL; and the necessity of —S–S– groups in insulin for hormonal activity have brought forth the importance of sulfhydryl groups in diabetes (see reviews of Houssay and Lazarow). According to Lazarow, the beta cells of the pancreas are rich in —SH groups, which are essential for the synthesis of insulin. Alloxan, by combining with these —SH groups or by oxidizing them, would inhibit insulin formation, and diabetes would ensue. The large number of papers published on the relation of thiols to hormone synthesis and hormone activity, although inconclusive, are indication of the existence of a fertile field for research.

Ionizing Radiations. The effects of ionizing radiations on aqueous solutions saturated with molecular oxygen are the biologist's main concern, since living cells contain about 80 per cent water, and are mostly aerobic. When water is irradiated by ionizing radiations (x-rays, alpha-rays, beta-rays, gamma rays) there are formed the free radical OH and atomic hydrogen:

$$H_2O \xrightarrow{\text{radiation}} H_2O^+ - e$$
$$H_2O^+ \longrightarrow OH + H^+$$
$$H_2O + e \longrightarrow H_2O^-$$
$$H_2O^- \longrightarrow H + OH^-$$

When molecular oxygen is present in water there occurs a stepwise reduction of this molecule by atomic hydrogen with the production of two oxidizing agents, the free radical O_2H, and H_2O_2:

$$O_2 + H \longrightarrow O_2H$$
$$O_2H + H \longrightarrow H_2O_2$$

Since sulfhydryl groups are easily oxidized, on irradiation of their aqueous solutions there may be the following oxidations:

$$2RSH + 2OH \longrightarrow RSSR + H_2O_2$$
$$2RSH + H_2O_2 \longrightarrow RSSR + 2H_2O$$
$$2RSH + 2O_2H \longrightarrow RSSR + 2H_2O_2.$$

Six to eight molecules of —SH compounds could be oxidized per ion pair produced on irradiation of water. Irradiation of glutathione produced the oxidation of 3.5 molecules per ion pair (onic yield $= \frac{M}{N}$, where M is the number of molecules reacting, and N, the number of ion pairs produced on irradiation of water) (Table 7). Similar results were obtained on irradiation of dithiols (Barron and Flood, 1950). The high ionic yield obtained is a demonstration of the extreme sensitivity of the sulfhydryl groups towards oxidizing agents. The role played by H_2O_2 can be determined by irradiation in the presence of catalase. Addition of catalase protected glutathione from oxidation by x-rays; from this protection it can be calculated that H_2O_2 contributed 20 per cent

TABLE 7

Oxidation of Glutathione by X-irradiation Effect of X-Ray Dosage

X-ray dosage	M	Ionic yield
r	x 10^{-15}	
500	3.39	4.13
1000	5.08	3.14
2000	10.16	3.14
3500	17.3	3.06
5000	26.62	3.29

1 cc. gluthathione, 2 x 10^{-7}M irradiated at 0°.
Phosphate buffer, 0.01M, pH, 7.3.

to the total x-ray oxidation. The inhibition of oxidation produced by the withdrawal of oxygen can be attributed to lack of formation of the O_2H radical, and of H_2O_2. Participation of these two agents amounted to 70 per cent of the oxidation in the presence of oxygen (Table 8). In agreement with these findings —SH en-

TABLE 8

Oxidation of Gluthathione by X-irradiation The Influence of Dissolved Oxygen

Gas in solution	Oxidation per cent
Air	25.9
Nitrogen	8.6

1 cc. of glutathione, 2 x 10^{-7}M. Buffer, 0.01M phosphate, pH, 7.0.
X-ray dosage: 10,000 r irradiated through closed vessels of plane-parallel pyrex glass of 1.2 mm. thickness.

zymes are very sensitive to ionizing radiation (Barron, Dickman, Muntz and Singer, 1949). They are inhibited by small doses of x-radiation, and this inhibition is abolished on addition of glutathione; if the inhibition goes beyond 50 per cent, reactivation is only partial, presumably because there is protein denaturation in addition to —SH group oxidation (Table 9). Addition of glutathione previous to irradiation completely prevented enzyme inhibition. This protection of —SH enzymes has been applied successfully by Patt and by Chapman *et al.* to the protection of animals against lethal doses of x-irradiation. Another evidence in favor of the sulfhydryl theory is given by the effect of x-irradiation on the respiration of sea urchin sperm and eggs (Bar-

TABLE 9

Inhibition of —SH Enzymes by X-irradiation. Reactivation by Glutathione Added After Irradiation

Enzyme	X-ray Dose r	Inhibition per cent	Reactivation per cent
Adenosinetriphosphatase	10	10	80
	100	27	97
	500	41	56
	1000	73	22
Phosphoglyceraldehyde dehydrogenase	100	21	complete
	200	50	62
	500	94	10

ron and Seki, 1952). The inhibition produced by relatively large doses of x-rays disappeared as the x-ray dose decreased, until there was increase of respiration (Table 10) exactly in the

TABLE 10

Effect of X-Rays and $HgCl_2$ on the Respiration of Sea Urchin Eggs (Unfertilized)

Inhibitor	O_2 Uptake in c.mm. Control	With Inhibitor
X-rays in r		
25,000	18.5	15.4
1,000	21.4	22
500	8.7	10.2
260	11.9	15.1
$HgCl_2$		
$5 \times 10^{-5} M$	12	24
$1 \times 10^{-4} M$	12	2

same manner as with —SH reagents (Barron, Nelson and Ardao, 1948). Chromosomes seem to possess few —SH groups in their molecules (Davidson and Lawrie, 1948). On oxidation by x-rays there would be molecular aggregation by the binding of two molecules through —S—S— linkages. Such bindings would also produce distortions and chromosome breaks. The inhibition of chromosome breaks by irradiation in the absence of oxygen (Thoday and Read, 1948) is evidence in favor of this suggestion.

I believe glutathione, cysteine, or one of the dithiols could be profitably used as a protective measure in cases of long exposure to x-rays, as in fluoroscopy, and on administration of large doses of x-rays.

Cell Division and Growth. Tribute is well deserved to the three investigators who discovered the role of sulfhydryl groups in cell division and growth: Rapkine, Hammett, and Voegtlin. The relation between thiols and cell division was formulated thirty-three years ago by Rapkine. He determined the glutathione content of sea-urchin eggs before and after fertilization, and at intervals after cell division. The glutathione content diminished soon after fertilization, reaching the lowest value 30 minutes after fertilization. There was then a sudden increase to reach a maximum 45 minutes after fertilization, *i.e.*, soon before cell division (Fig. II).

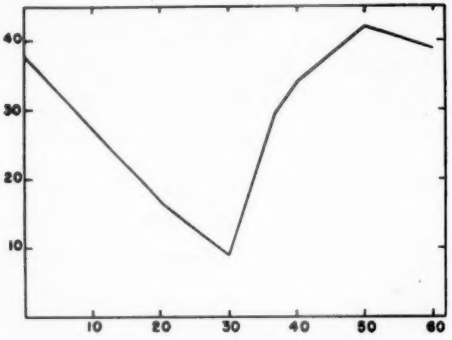


FIG. II. Sulfhydryl content of fertilized sea urchin eggs (43). Abscissa, time after fertilization in minutes. Ordinate, —SH content in mg. per 100 g. eggs (fresh weight).

Addition of small amounts of $HgCl_2$ to fertilized sea-urchin eggs stopped mitosis completely. Mitosis started again as soon as cysteine or thioglycolic acid was added. Moreover, on measuring the —SH groups in a protozoan *(Gymnodinoides inkystans)*, Rapkine found that they were all of the sluggish type (negative nitroprusside test). At the moment of cell multiplication the nitroprusside test became positive and the sulfhydryl content in the cell increased steadily, reaching its maximum soon before cell division. Hammett and coworkers in experiments on organ regeneration, embryonic development of marine organisms, and growth of seedlings and algae, have given evidence that the sulfhydryl concentration increases during development and that

this process can be inhibited reversibly by treatment with mercaptide-forming heavy metals. Similar observations were made by Voegtlin and coworkers, who also found in experiments with *Amoeba* and *Paramecium* that glutathione increased the rate of volume change in the nucleus, and that mercaptide-forming compounds, such as the salts of Au, Cu and trivalent As, inhibited this effect. In seedlings, at the very beginning of germination, there is an increase in the glutathione content, which rapidly reaches a maximum (Hopkins and Morgan, 1943). Brachet has discussed extensively the possible mechanisms of the effect of —SH groups on cell divisions. It is quite likely that the enzymes for the synthesis of ribose and desoxyribose nucleic acids are —SH enzymes of very labile —SH groups. An increased concentration of glutathione would be required not only to keep these enzymes in the reduced —SH state, but also to shift the metabolism of the cell towards synthesis.

The role of sulfhydryl groups in the growth of tumor tissues is a corollary of the function of these groups in cell division and cell growth; it is to be expected, as has been often found, that the —SH group content of tumor tissues would be greater than that of non-tumorous tissue. The same increase in —SH groups was reported by Binet and Magrou on inoculation of *Bacillus tumefaciens* (a tumor-producing microorganism) to the stem of certain plants. The glutathione content was greatly increased in the stem and in the leaves during the period of growth of the tumor. Glutathione disappeared from the tumor during necrosis.

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RADIOAUTOGRAPHY IN CYTOLOGY*

PATRICK J. FITZGERALD

The radioautographic technic which permits the localization of extremely small amounts of radioactive isotopes in biological specimens has been recently extended to the cytological level and should be of considerable interest to those engaged in a study of cellular metabolism. The chief impetus to a cytologic application has been the development of new photographic emulsions which are now so sensitive that individual electron tracks coming from an organism may be recorded (King, Harris and Tkaczyk, 1951) or, with non-track emulsions and some isotopes, possibly as few as 100 radioactive atoms per cell may be recognized (Lajtha, 1952).

Paramecia have been studied in our laboratory and have shown incorporation of many labeled compounds. C^{14}–labeled glycine, formate, acetate, methionine and nucleic acids precursors as well as S^{35}–labeled methionine have been demonstrated intracellularly in both killer and sensitive strains of *P. aurelia* (Hamilton and Fitzgerald). With the same organism it has been shown that tritium (H^3) is concentrated in various intracellular regions (Eidinoff *et al.*, 1951; Fitzgerald, *et al.*, 1951). This has been confirmed by Chapman-Andresen *et al.* (1953) who had previously used C^{14}–labeled compounds to demonstrate the distribution of food from its early localization in vacuoles to its later uniform distribution throughout the organism (Andresen *et al.*, 1950). Beta particle tracks of P^{32} from *P. caudatum* (King *et al.*, 1951) and single tracks from Colpidium have been recorded (Harris *et al.*, 1950).

We have shown cytoplasmic distribution of tritium with *Torula utilis* which was grown in a medium containing H^3–labeled water and sodium acetate (Eidinoff *et al.*, 1951; Fitzgerald *et al.*, 1951). Pathogenic fungi labeled with P^{32} and I^{131} were relatively indestructible in the dog as compared with nonpathogenic fungi when the radioautographic technic was used

* From the Pathology Laboratories and the Division of Physics, Sloan-Kettering Institute, Memorial Center for Cancer and Allied Diseases, New York 21, N.Y. Supported in part by grants-in-aid from the United States Atomic Energy Commission (No. AT (30–1) 1451).

to determine the presence in the lesions of the two types. Tubercle bacilli also incorporated isotope which was demonstrated in the tubercular lesion (Pearson *et al.*, 1949). Algae (Nitella and Chara) have been studied with P^{32} (Mullins, 1947).

Individual blood and marrow cells have concentrated enough radioactive isotopes to give discernible autographs (Boyd *et al.*, 1950; Lajtha, 1952; Morse, 1950). C^{14} tracks have been described as originating in liver cells (Boyd and Levi, 1950). The cells of the reticulo-endothelial system collect many substances and we have demonstrated radioactive particles of gold (Au^{198}), thorotrast (Th^{232}) (Fitzgerald, 1952) and silver (Ag^{110}) (Fitzgerald) in individual cells. Pelc and workers have demonstrated the localization of P^{32} and S^{35} in chromosomes of the bean root, *Vicia faba* and attempted to relate their findings with the synthesis of desoxyribonucleic acid and nucleoprotein (Doniack, 1953).

Of particular interest to us has been the question of the relative incorporation of labeled compounds by tumor and embryonic cells grown simultaneously in a medium containing the radioactive isotope. We have used radioautography to determine the relative concentration of nucleic acid precursors in mouse tumor cells and embryonic AK mouse skin cells. A preliminary report of the technic and results has been made (Fitzgerald, 1953). Pelc and Spear had previously shown that chick fibroblasts grown in a medium containing P^{32} showed appreciable concentration of the isotope in the cytoplasm and low concentration in the nuclei and vacuoles of resting cells (Pelc and Spear, 1950). Our technic and results will be summarized.

Technic

Tissue Culture. The neoplasm used for most studies was the mouse sarcoma T–241 grown in C 57 black mice. Ten days after transplantation the mouse was killed by severing the spinal cord and small pieces of the tumor transferred to a sterile petri dish and cut with sterile instruments into small pieces averaging about a millimeter in diameter (Fig. I, A and B). Pieces of embryonic skin were taken from AK mouse foetuses at a stage about two thirds of the gestational period. Sarcoma 180 tissue was also used, removed from subcutaneous AK mouse transplants 7 days after inoculation.

Three pieces of tissue were transferred to a small rectangular No. 1 cover slip about 2.0x0.5 cm. They were covered with a few drops of adult rooster plasma and then clotted with a few drops of embryonic extract (Fig. I, C).

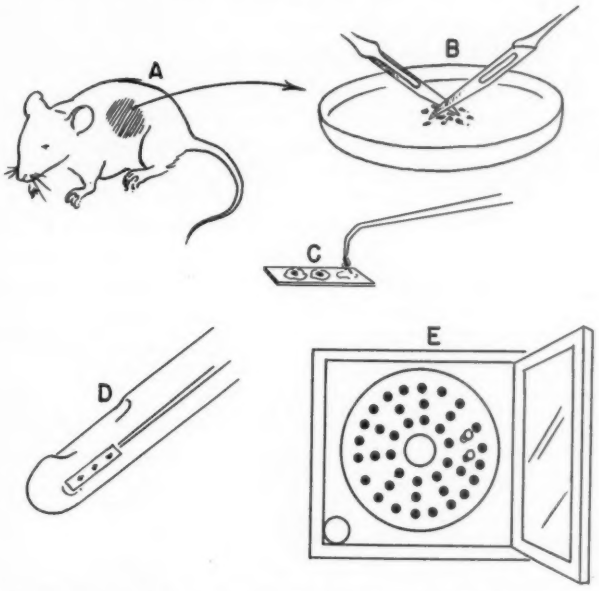

FIG. I. Preparation of mouse tumor transplant (A) for tissue culture in roller tube. Control treated similarly and placed in same tube (D) as tumor.

The cover slip was then placed in a wall recess in a roller tube and covered with the medium (Fig. I, D). The standard medium No. 212, was composed of 2 parts of Gey's solution, 1 part of embryo extract and 2 parts of serum. One ml. of the medium was used for each test tube which contained the two coverslips and cultures. To each ml. of the medium was added 25 micrograms of Streptomycin and 25 units of Penicillin G. The test tubes were placed in a roller mechanism which rotates 6 times per hour and incubated for 48 hours at 37.5° C. (Fig. I, E).

The tissues were then dosed by removing 0.1 ml. of the medium and replacing it by an 0.1 ml. of the compound to be tested.

The adenine and 2,6–diaminopurine solutions contained 1 micromole per ml. The activities of the C–14 adenine and C^{14}–2,6–DAP were 10,000 counts per minute per microgram.[1] When inactive adenine and C–14–2,6–diaminopurine were both added to the medium 0.2 ml. of the medium was withdrawn and replaced by 0.1 ml. of each.

The tissue cultures are incubated at 37.5° C. for 24 hours in the roller tubes containing the labeled compounds.

After incubation the cultures were rinsed twice with physiological saline and the clots stripped under the dissection microscope (Fig. II, A). They were fixed, dehydrated, embedded in

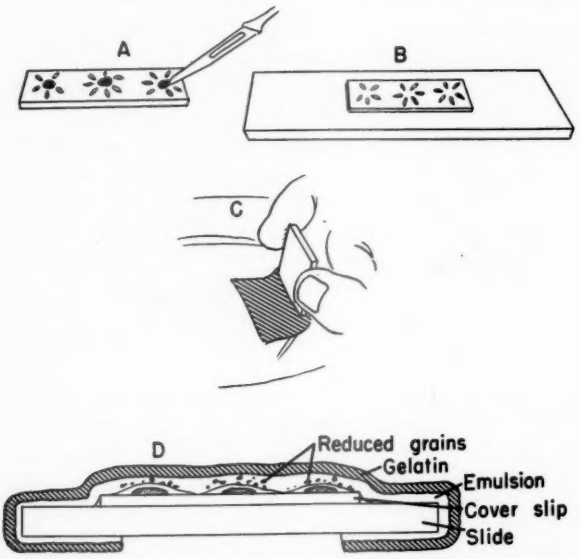

FIG. II. Removal of central clot (A) for fixing and histologic sectioning. Cells that have grown out from the clot are fixed in situ and may be stained, subjected to enzyme digestion, counted for radioactivity or exposed to solvents (B). Both sections of the clot and the whole cells, untreated or treated, may now be covered with stripping film (C and D).

celloidin and later in paraffin. They were sectioned at 1.0 or 0.1 micron, floated on a previously prepared glass slide, the embed-

[1] Kindly prepared by Dr. G. B. Brown, and Staff, Sloan-Kettering Institute.

ding material removed, and preparation was ready for the application of the radioautographic stripping film (Fig. II, C and D). The cells that had grown out from the periphery of the clot (Fig. II, B) were fixed on the cover slip, subjected to enzyme digestion or to chemical solvents, stained, counted by the Geiger-Mueller technics, or were covered, unstained by stripping film.

Radioautographic. The emulsion used, the Kodak Ltd., Autoradiographic Stripping Plate, is a stripping film composed of 5 microns of emulsion on a 10 micron gelatin base which is attached, emulsion side up, to a glass plate. The technic of using the stripping film has been described in detail (Doniak, 1953; Fitzgerald, 1953). Essentially, the film is stripped off the glass plate, floated emulsion surface down in a water bath, and then the histologic section of the plasma clot or the peripheral cells on a cover slip mounted in a glass slide are immersed beneath the floating emulsion. Upon withdrawal of the specimen the emulsion drapes itself over the preparation (Fig. II, C). After exposure and processing the preparation appears as in figure II, D. The cells or section may be stained before radioautography or through the permeable gelatin base after radioautographic processing. We have stained some slides before autography by the Feulgen technic and most slides after photographic processing with the cochineal stain (Fitzgerald, 1953).

Grain Counts. Since the number of grains reduced in the photographic emulsion overlying the cells is proportional to the amount of ionizing radiation absorbed, grain counts give the relative amounts of isotope present in the two similar preparations containing the same isotope. These were performed by visible light microscopy using a 90x oil immersion fluorite objective and a 10x ocular. All cells showing evidence of radioactivity were counted. Grains above each cell were counted (Fig. II, D) and background grains subtracted from the cell counts. The average number of grains per tumor cell and per skin cell was determined. Bar graphs were made by plotting the number of cells against the number of grains in decades per cell to determine the distribution of radioactivity.

Results

Our grain counts show that the average number of grains reduced per tumor cell was greater than the average number

reduced by the skin cells when both were incubated in C^{14}–labeled adenine.

With C^{14}–labeled 2,6–diaminopurine the average number of grains per cell was about the same in the tumor and embryonic cells but there were more cells in the tumor group with high counts. In the tumor explants it was seen that fibroblasts, monocytes, and other cells were associated with lower grain counts than were the obvious tumor cells which generally were associated with high counts. As determined by the larger exposure period required in this group to produce an autograph the concentration of 2,6–diaminopurine was much less in both the skin and tumor cells than was the adenine.

When inactive adenine was added to a medium containing C^{14}–labeled 2,6–diaminopurine the incorporation of the latter in tumor and skin cells was greatly inhibited as compared to similar cells exposed to the C^{14}–labeled 2,6–diaminopurine alone. Tumor cells on the average concentrated more C^{14}–labeled 2,6–diaminopurine in the presence of adenine than did the skin cells.

Radioautographs of histologic sections of tumor and skin cells cut at 1 or 0.1 micron showed that the isotopes were intracellular and were present both in the nucleus and in the cytoplasm in all cases. Use of hot trichloracetic acid, desoxyribonuclease, ribonuclease, and Feulgen staining before radioautography reduced the number of grains reduced per cell as compared with similar preparations not subjected to such procedures. For these reasons we believe that our isotopes were incorporated, in part, in the nucleic acids, both in the nuclear desoxyribonucleic acid and in the cytoplasmic ribonucleic acid.

Summary. Some of the uses of radioautography in cytology have been pointed out. It is believed that the method has even greater potentialities. An illustrative application is given in detail—the use of the method in determining the relative concentration of labeled nucleic acid precursors by neoplastic and embryonic cells grown in a medium containing a labeled compound. Our findings lead us to believe that we have demonstrated the incorporation of the precursor into the nucleic acids of the nucleus and cytoplasm of the cells.

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THE CONTROL OF HUMAN CANCER BY STERIOD MODIFICATIONS OF THE INTERNAL ENVIRONMENT

CHARLES HUGGINS*

It has been said recently (Haddow, 1952), "Of all the branches of physiology and chemistry having a direct bearing on the cancer problem, steroid endocrinology holds a key position, and is certain to figure prominently—possibly decisively—in the ultimate solution." Steroids, synthetic and natural, are known to induce a great variety of cancers in experimental animals. Also many kinds of experimental cancers can be prevented by appropriate use of steroids. Further, changes in the content of steroids in the body can force certain cancers to regress or recede with concomitant important improvement of the human host. Evidently steroids of various types are of significance in the causation, prevention and treatment of cancer.

Two new principles of medicine can be enunciated from the studies to be described. First, cancer is not autonomous in its growth with intrinsic self-perpetuating properties. Certain cancers retain sufficient of the characteristics of the normal cells from which they arose that the neoplastic cells possess the functions of the normal cells. Secondly, disease, in this case cancer, can be sustained and propagated by hormonal activity which is not necessarily exaggerated in rate but which operates at normal or even subnormal levels. Under this principle the importance of trace amounts, or small quantities of steroids in the activation of cancer may be appreciated. These principles are the basis of the control of cancer by physiologic means—of benefit secured by alteration of the tumor-host relationship.

There are two general methods of approach to the physiologic control of cancer by steroids. Certain normal cells and their cancers require hormonal support for maximal activity and they pass into a sluggish metabolic state when deprived of this stimulating factor; examples of cells affected by hormonal control

* From the Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois. The Bertner Lecture given at the Seventh Annual Cancer Symposium, May, 1953, at the M. D. Anderson Hospital for Cancer Research, Houston, Texas.

through deprivation are prostatic and mammary cells. In the second method of steroid control excessive hormonal pressure is applied to the cell which figuratively "burns up in the flames." An example of the second technique is the modification of activity of the lymphocyte and its neoplasms. Through the work of Dougherty and White (1943) it was learned that the pituitary protein ACTH causes a decrease of size of lymph nodes in intact rodents; Heilman and Kendall (1944) then showed that cortisone caused the quick dissolution of lymphosarcoma in the mouse; Olaf Pearson and colleagues (1949) then demonstrated that cortisone could cause significant, if temporary, regression of lymphosarcomas and certain leukemias in man.

Fifteen years ago the physician treating people with advanced cancer was concerned chiefly with methods to reduce pain and to decrease the odor of sloughing tumors—tasks which kept him busy indeed since these cases were frighteningly common. Surgery and irradiation were the only treatments available before 1940 and they could only provide some palliation of the disease chiefly through the relief of obstructions and the control of hemorrhage. When life was prolonged by the methods available, it was commonly not good life; days or a few months were added only to permit the person with cancer to taste to the full the misery of the slow agonizing death of his disease.

During these 15 years therapeutic advances have been made by the experimentalists. It is true that no complete therapy has been devised for all of the neoplastic disease; for that matter cures have not been devised for all of the infectious diseases where medicine has made its greatest progress in recent times. However, advances of significance in practice as well as theory have been made in certain kinds of cancer. Although nothing more than partial solutions have been achieved they have been worthwhile to the patient. The aims of medicine are the relief of suffering and the prolongation of *good* life or as Francis Bacon wrote, "To make imperfect men comfortable." These therapies have emerged from laboratories as have all great advances in medicine in this century. The treatments have indicated that the successful treatment of cancer by pills is possible. This principle having been established only time, laboratories and an enlightened personnel are needed for further advances in the treatment of human cancers by medicines.

Only steroid therapy can be considered at this time. As chemotherapeutic agents steroids have several important properties. They are not toxic and if they do no good they wreak no great or sudden harm to the patient with cancer. Also, many of them are active in small or trace amounts. Further their relatively poor solubility in body fluids furnishes long, uninterrupted physiologic action.

This essay is concerned for the most part with prostatic and mammary cancers because more is known about these tumors than any others; they happen to be among the commonest of the cancers. The development of the treatment of these growths by steroid modifications was not empirical; it was deduced from a continuous series of laboratory observations which were carried out, very slowly. The results by good fortune happened to have a sharp focus.

The control of cancer of the prostate by steroid means emerged from experiments on the prostate of dogs and the first goal was a simple quantitative assay technique. The prostatic secretion of the dog was collected at frequent intervals for months or even years, the experimental animals remaining in good health and the effects of the administration or the deprival of steroids on the output of prostatic fluid was determined. In brief, androgenic steroids initiate and maintain prostatic secretion whereas anti-androgenic agencies such as orchiectomy or estrogen administration suppressed prostatic activity.

It has been said that in any successful experiment there is an element of luck. The good fortune in this work was the selection of the dog as the subject; this species is the only one other than man to develop spontaneously tumors of the prostate. It was inevitable that sooner or later old dogs with these lesions should be encountered. As a matter of fact, after a short period of vexation because the lesion was interfering with a physiological study, a search was made specially for dogs with this condition.

It was observed that anti-androgenic measures caused a rapid regression of this canine neoplasm whilst androgen increased its size. It was obvious that these effects must be investigated with respect to the malignant and the benign tumors of man, and distinct effects were observed in both conditions, but were more obvious in prostatic cancer.

Now, in science the method of proof is often of greater interest than that which is proven. The effects on disseminated cancer of the prostate were proven by chemical means, namely through a study of the acid and alkaline phosphatases of serum. Acid phosphatase, a component occurring in human prostatic epithelium in rich concentration, had been found, by Gutman and Gutman in 1938, to be increased in the blood serums of certain patients with disseminated prostatic cancer. Now prostatic cancer frequently metastasizes to the bones, in which tissue it flourishes, evoking an overgrowth of the osteoblasts, and Kay had shown in 1929 that when the osteoblasts are stimulated the alkaline phosphatase of serum increased. We could then make a synthesis of these facts (1941) and study the course of the disease by determining the levels of these enzymes at frequent intervals; acid phosphatase indicated the activity of the malignant cells, while the quantity of alkaline phosphatase indicated the reactivity of non-malignant tissue which was the host of the cancer. In brief, the administration of testosterone increased the activity of the tumor, and anti-androgenic measures caused a decrease of acid phosphatase toward or to normal. Also when the lesion was brought under control there was at first a rise of alkaline phosphatase, indicating a stimulation of osteoblasts (frequently due to a filling in of the bone lesions in the healing process) with a return to normal after some weeks.

The results of anti-androgenic control are of interest from several standpoints. The effect of control is often catastrophic to the tumor and of course beneficial to the patient. There occur an increased appetite and intake of food, and relief of pain. Protein synthesis begins at a rapid rate. The vicious circular chain is broken and the invalid is relieved. Now the striking feature about all of this is its speed; improvement may be detected by chemical means within 24 hours.

The minimal effective dosage of stilbestrol needed to bring about control has been determined; it is between 0.25 and 0.3 mg. a day of diethyl stilbestrol administered intramuscularly, thus establishing the fact that control lies within the physiological limits. Diethyl stilbestrol happened to be the first non-radioactive substance which was found effective against widespread cancer in man.

Statistically, about 95 per cent of patients with prostatic cancer obtain some relief, which is usually considerable. However, a relapse occurs in about 75 per cent of the patients after 6–18 months and these we designate as the failure cases. On the brighter side 20 per cent of patients in our first series are alive and in good health after 10 years, without evidence of disease by chemical and clinical methods. In these people the control certainly has been fairly extensive and moderately long.

In the failure cases certain factors may be discerned. (1) In some of those who relapse there is production of androgen in extra-genital depots which have been identified as the suprarenal glands. (2) Certain of the cancer cells acquire or retain the property of androgen-independence. We conceive of control as the withdrawal, in effect, of androgen from certain cells which require it as an essential nutritive element. Some of the prostatic cancer cells find ways of maintaining themselves in the complete absence of androgen so that escape from control is inevitable.

In recent years our laboratory has been concerned with the elimination of steroids of adrenal origin, and techniques have been devised for removal of the adrenal glands with hormonal substitution to maintain life, since otherwise adrenalectomy is fatal in man within a few hours. In 1945 the first adrenalectomy was carried out and a patient survived four months. The chief steroid used for maintenance was desoxycorticosterone. While there was some regression of the prostatic cancer after removal of the adrenals, life was not good since the patient had evidence of adrenal insufficiency.

With the advent of an adequate quantity of cortisone, in 1950, the problem was again considered. Relatively simple surgical and medical techniques were devised so that adrenalectomy can now be carried out with little mortality or morbidity. The adrenalectomized patient maintained on 11–oxygenated steroids presents the picture of excellent health and is able to engage in all of his customary activities. He is in constant danger however of developing adrenal insufficiency when the substitution therapy is inadequate. Replacement is generally satisfactory when cortisone acetate 37.5–50 mg. daily is given by mouth and the care of adrenalectomized patients is very simple.

The chief application found for removal of adrenal steroids by excision of the adrenal glands is mammary cancer of human males and females. Certain cancers of the breast, in both men and women may be composed of cells of sufficient functional maturity that they will shrink when supporting hormones are abolished through removal of their source; the patient is benefited in this circumstance. Sir George Beatson (1896) was the first to show that cancer of the breast sometimes recedes when the ovaries are removed; this work was done before there was any concept of endocrinology. Subsequent work has shown that there are however dual sources of estrogenic hormones, the ovary and the adrenals. These glands produce hormones of ovarian type in varying ratios, although commonly after the menopause the ovarian component is greatly reduced in quantity. According to the extent to which ovary and adrenal respectively contribute to the endocrine pool, the patient is improved by ovariectomy, adrenalectomy or the combined removal of both structures. The removal of ovaries and adrenals, we find, eliminates estrogen from the urine.

Following the removal of the appropriate source, the patient with dependent mammary cancer is relieved of pain; there is a decrease in size of the primary tumor and its metastases; osseous lesions calcify and osteolytic lesions and pathological fractures heal; the accumulation of fluid due to cancer in the pleural or peritoneal cavity ceases; masses of cancer in the liver and brain have been known to decrease in size. The diminution in activity and size of the cancer causes improved appetite, gain in weight, recovery from anemia and relief of discomfort. The beneficial results from adrenalectomy are still present in the first patient subjected to adrenalectomy for mammary cancer, now more than 2 years ago.

Regression of mammary cancer after deprivation of steroids is a function of removal of critical amounts of sustaining hormones together with sufficient maturity of the neoplastic cells that they are dependent on hormones—it rests on hormone production plus reactivity of cells. Mammary adenocarcinoma usually fulfills the latter requirement and so constitutes a special category of breast cancer contrasted to ductal or undifferentiated cancer. We consider that the formation of the acini in

mammary adenocarcinoma is a postive process directly attributable to secretion induced by hormones. In our theory the lumen of the acinus cannot be empty—it contains secretion; otherwise it would be filled by the ingrowth of cells as is the lumen in ductal carcinoma. Many of the cancers derived from the lobule-alveolar apparatus of the breast are hormone dependent in contrast to those arising from ducts. The elimination of critical amounts of supporting hormones from the internal environment of certain cancers alters cell metabolism sufficiently to stop secretion causing the acini to shrink and the activity of the cancer cells to decrease with improvement of health of the patient. Control replaces lack of control.

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AMINO ACID METABOLISM OF NEOPLASTIC TISSUES AND REGULATORY MECHANISMS

SAUL KIT*

The fate of amino acids after they enter normal or neoplastic cells may be illustrated by the case of glumatic acid (Fig. I). It may be incorporated into proteins, glutathione, or other nitrogenous substances, or it may be transformed to alpha ketoglutaric acid. In the latter case, the carbon of glutamic acid may be burned to CO_2 and water by the enzymes of the tricarboxylic acid cycle, or depending on the circumstances, utilized partly for the formation of fats and carbohydrates.

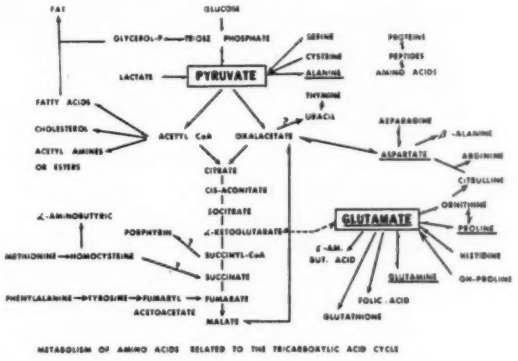

FIGURE I.

Most biochemical reactions are reversible so that glutamic acid may also be formed from carbohydrate precursors; or, it may be formed from certain other amino acids. As Barron (1942), and more recently Potter and Heidelbarger (1950), have pointedly emphasized, many other tissue constituents are also metabolized through multiple metabolic pathways.

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* From the Protein Section, Department of Biochemistry, The University of Texas, M. D. Anderson Hospital for Cancer Research, Houston, Texas. Presented at the Seventh Annual Cancer Symposium, May, 1953, at the M. D. Anderson Hospital for Cancer Research, Houston.

In cancerous tissues, the overall flow of materials is along such pathways as may lead to continued and autonomous growth. One manifestation of this is embodied in the concept of the tumor as a nitrogen trap (Mider, 1951). Thus, although reversible dynamic equilibria exist, the equilibria are on the side of retention of nitrogenous substances by the tumor, once such substances enter the cell. The question therefore arises as to what may be the regulatory mechanisms which govern the direction of flow of cellular metabolites. In the discussion which follows, only a few of these mechanisms will be mentioned.

The extent to which a metabolite will be utilized in one particular reaction depends, firstly, on the concentration of that substrate in the cell. The velocity of enzymatic reactions increases with substrate concentration until a given enzyme is saturated with substrate. It is to be doubted that most substrates are present at optimal concentrations in the cells of living organisms so that variations in the availability can have rather large effects upon the velocity of reactions.

Also, since a number of enzymes may be competing for the same substrate, the extent to which one pathway is followed depends upon the relative affinity of each enzyme for that substrate, the accessibility of the substrate to the enzyme, and the concentration of each holoenzyme in the cell.

From this it follows that dissimilar metabolic functions may be pursued by different cells having the same kinds of enzymes provided that the relative amounts of competing enzymes and enzymes which act in sequence to one another are changed. This point of view stresses the *pattern* of enzymes in a cell (LePage, 1950), and implies the functional inhibition of one enzyme by another through competition for substrate.

It is reasonable to assume that the endogenous substrate concentration at a given time reflects the enzymatic pattern of the cell. Therefore, if the enzymatic *pattern* of the cancer tissue differs from that of the homologous normal tissue, the *pattern* of substrates within the cancer cell will also differ. Moreover, observations of substrate patterns can provide insight into the nature of the enzymatic pattern, or suggest experiments relevant to the elucidation of that pattern.

With these considerations in mind, the concentrations of a number of amino acids have been measured in various tissues by

means of paper chromatography (Awapara, Landua, and Fuerst, 1950; Kit and Awapara, 1953). It was noted that each tissue had a distinctive pattern (Fig. II). Thus, thymus is particularly

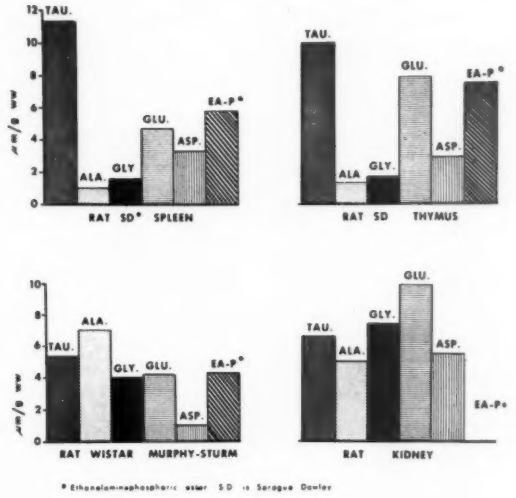

FIGURE II.

rich in taurine and ethanolamine phosphoric ester; kidney is very poor in ethanolamine phosphoric ester and in glutamine, liver can be distinguished from kidney by its high glutamine content, etc. All lymphatic tissues so far examined from rabbits, two strains of mice, and two strains of rats, have very similar amino acid patterns but this pattern is quite different from that found in lymphosarcomas, figure III. The patterns of four different lymphosarcomas were found to be very similar; and they were in turn different from that of other tumors, as for example, a rat carcinosarcoma.

Alanine and related substances. Lymphosarcomas have relatively high concentrations of alanine and glycine and lowered levels of aspartate and ethanolamine phosphoric ester. Alanine also appears to be elevated in the carcinosarcoma.

The high alanine concentration in tumors perhaps may be attributable to the capacity of such tissues to concentrate alanine

from the blood (Christenson, 1952).[1] However, alanine can also be formed rapidly in lymphosarcomas (Kit and Greenberg, 1951). Figure IV illustrates an experiment in which isotopic lactate was incubated with normal spleen cells and lymphosarcoma cells. It will be noted that considerably more radioactivity was found in tumor alanine than in spleen alanine.

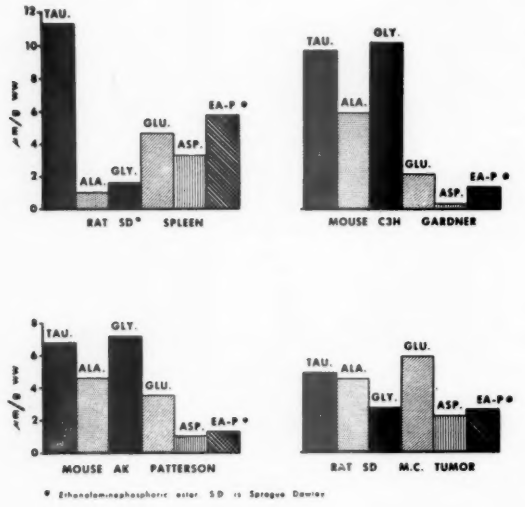

FIGURE III.

Measurements in a number of tumors indicate that the lactate concentration is relatively high (LePage, 1948). A few measurements have been made of keto acid levels in some tumors and normal tissues. Pyruvate and ∝-ketoglutarate may be elevated while oxalacetate is very low in all tissues (LePage, 1950; Frohman *et al.*, 1951).

The position of alanine and its precursor, pyruvate, on the metabolic map is shown in figure I. It would seem that all of these observations are related to the classic experiments of Warburg in which it was found that tumors have a very high rate of glycolysis. The Gardner lymphosarcoma for example, has a

[1] Similar considerations apply to the other metabolites.

$Q^{N_2}{}_A$ [2] of over 50 (Kit, Bacila, and Barron, publication pending), while rat thymus and spleen slices were reported by Greenstein (1947), to have a $Q^{N_2}{}_A$ of 8. The maximum observed glycolysis of rabbit appendix cell suspensions was 25, a very high rate for a normal tissue (Kit, Bacila, and Barron, publication pending), though only half the rate of lymphosarcoma cell suspensions.

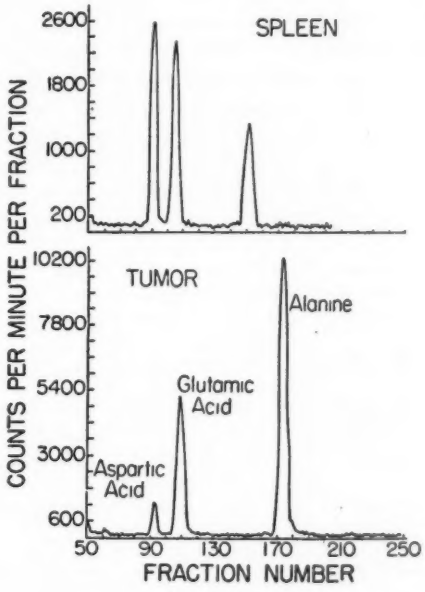

FIGURE IV.

Presumably, alanine formed in the cell arises from pyruvate by transamination. To investigate this point, various transaminases were measured in lymphatic tissues (Kit and Awapara, 1953). Appreciable alanine-α-ketoglutaric transaminase activity was observed in both normal and malignant lymphatic tissue (Table 1). It will be noted that the aspartic-glutamic system is considerably more active. When the reaction of pyruvate with glutamate or glutamine was measured, however, only very weak

[2] Anaerobic glycolysis in terms of mm^3 lactic acid equivalent/mg. dry tissue/hr.

activity was found. Efforts to demonstrate a reductive amination of pyruvate were unsuccessful. The significance of these latter results are as yet unexplained.

Amino acids related to tricarboxylic acid cycle. The proline concentration of lymphosarcomas is very high and glutamine, rather low in normal lymphatic tissues, is further reduced in the tumors. Also, it will be recalled, low levels of aspartate were found in the lymphosarcomas and apparently little oxalacetate is present in most tissues.

After *In vitro* incubations with labelled lactate, little radioactivity was found in the aspartate of tumor, although appreciable radioactivity was present in spleen cell aspartate (Fig. IV). These observations are of interest since one might infer that aspartate is not formed in tumor cells. This is unlikely since as shown previously (Table 1), aspartate may be formed very

TRANSAMINASE ACTIVITY OF LYMPHATIC TISSUES AND TUMOR

μ moles amino acid formed per gram wet weight tissue in 30 min.

TISSUE	ALANINE AKG	ASPARTIC AKG	ALANINE OAA	GLUTAMIC OAA
THYMUS WISTAR	15	68	---	208
SPLEEN WISTAR	27	107	14	298
SPLEEN C3H MOUSE	13	158	21	268
APPENDIX RABBIT	41	70	5	188
MURPHY- STURM LYMPHOSARCOMA	13	195	2	317
MECCA LYMPHOSARCOMA	58	198	12	255
GARDNER LYMPHOSARCOMA	38	136	29	286

AKG and OAA are alpha-ketoglutaric and oxalacetic

TABLE 1

rapidly in lymphosarcomas by transamination, if oxalacetate is *added* to the system. The reverse reaction is equally rapid. Recently, it was also possible to show that radioactive aspartate can be formed by lymphosarcoma cells from labelled glutamate or succinate at rates comparing favorably with those of suspensions of rabbit appendix cells (Kit and Awapara, 1953). Furthermore,

although little free aspartate is found in lymphosarcoma, considerable amounts of aspartate may be released upon acid hydrolysis of the protein free extract. It therefore seems likely that aspartate is formed but rapidly utilized in anabolic reactions.[3]

Glycine metabolism. It will be recalled that the glycine concentration is very high in lymphatic tumors. This is of interest because of the multiple anabolic reactions in which glycine participates. The concentration of ethanolamine phosphoric acid, on the other hand, was low. The relationship between glycine and ethanolamine phosphoric ester is shown in figure V.

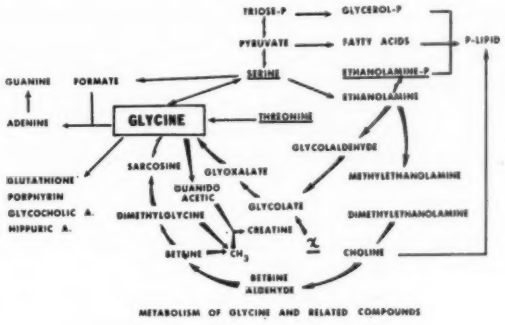

FIGURE V.

Preliminary experiments indicate that the threonine concentrations are high in the lymphatic tumors. Possibly, a block in serine decarboxylation occurs in the mouse tumors. Further experiments will be required to test these and other possibilities.

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THE QUANTITATIVE ANALYSIS OF THE CELL BY INTERFERENCE AND ULTRAVIOLET MICROSCOPY

ROBERT C. MELLORS*

This discussion will describe recent developments of a method of microscopy called multiple-beam interference microscopy and present applications of this method in cell research. A citation of the literature on this subject includes but a couple of dozen contributions, notably those by Tolansky, Frederikse, Merton, Richards, Barer and others and those from our laboratory. The plan of the presentation is first to give a short description of the underlying physical principles and then to show how it is possible by a non-destructive optical method to measure in absolute units a number of structural properties of the cell. Among these are the hydrous mass and the organic mass of the whole cell, the cytoplasm and the nucleoplasm and the organic mass of chromosomes.

Theory and methods. In the customary perception of light energy, whether by vision or by photographic, photoelectric or other radiometric devices two properties of a light wave are utilized: the frequency (or color) and the amplitude (or intensity). An additional property of the light wave, namely, the phase of the wave, customarily escapes recognition. However, the interference microscope and the more familiar phase microscope analzye the phase, or the oscillatory position, of the wave motion and as a consequence permit the visualization of what, in the customary light microscope, is a transparent cell object. The reason for this is that a light wave even in its transit through a completely transparent object encounters difficulty and is

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slowed down by almost all of the molecules through which it passes. The net result of this is that a wave which passes through a cell may emerge so that its oscillation is at the top of the wave motion, whereas, a wave which passes around the cell—that is, through the mounting medium—may emerge at the bottom of the wave motion or at some intermediate position. Thus, the two waves are out of phase; one has a phase displacement or retardation with respect to the other; and waves bearing such a relation, when appropriately combined, interfere with each other. One can readily perceive that if the top of one wave motion is added to the bottom of another, each neutralizes or cancels the other, the resultant amplitude decreases and the intensity diminishes. In this manner a cell which produces a change in the phase of a light wave appears in contrast, either dark or bright in relation to the surrounding medium, when viewed with an interference microscope.

However, it is necessary that this interference, which I have described, be repeated over and over again so that practically all of the bundles of light waves are involved in the process. In order to facilitate this, the cell is mounted between a glass slide and coverslip which are in no way unusual except that one surface of each (that nearest the object) is coated like a mirror with a reflecting metallic surface. The effect of this is to transmit the waves back and forth many times through the object before they emerge and are picked up by the conventional microscope objective and brought to focus. It is possible to mount the cell either in air, which is actually saturated with water vapor, or in an aqueous medium. The appearance of the interference pattern and the type of information obtained is quite different in these two circumstances.

The multiple-beam interference pattern of the living cell mounted in air (such as that of each of these two sarcoma cells growing side by side in tissue culture) consists of several, more or less, elliptical bright interference rings or fringes. The fringes, in general, are concentric with the center of the cell and are symmetrical, but they become more irregular at the periphery of the cell. These fringes signify changes in the phase of the light wave, and it can be shown that they define loci of identical optical path lengths or, in the case of optical homogeneity, loci of

identical thicknesses of the object. Thus, the interference pattern provides not only the lateral dimensions of the object but also a third, or a thickness, dimension. This, then, constitutes three-dimensional microscopy.

An interpretation of the interference pattern in air and a construction of the thickness-contour of a hypothetical cell which has four interference fringes are shown in this figure. A certain thickness of protoplasm is sufficient to produce light wave interference, and this thickness is repeated several times. The area of an interference fringe and this thickness, readily computed for a particular wavelength, define cylindrical solids with volumes, such as, v_1, v_2, etc. The volume of the cell, especially that of a living cell in tissue culture which has one plane surface parallel to the supporting slide, is the envelope of these cylindrical volumes and, as a first approximation, may be taken to be equal to the sum of these volumes. Thus, the volume of the cell is equal to the summation of the fringe areas multiplied by the thickness increment, or altitude, which for the monochromatic green line (λ,0.546μ) is computed to be 0.82μ. Furthermore, if the specific gravity of the cell is taken as a first approximation to be unity, the volume is readily converted to the mass which is called the hydrous mass. Interferometry in air provides, therefore, the thickness, volume and hydrous mass of the cell, and the results are expressed in centimeters, cubic centimeters and grams, respectively.

The multiple-beam interference pattern of a living cell mounted in an aqueous medium displays, in general, an interference fringe which has an extended area that may appear bright on a dark background, or dark on a bright background, as shown here for embryonic fibroblasts. Such an extended area defines a locus of identical length of the optical path and of identical phase displacement by the object. In this case, the phase displacement is assigned to, and is proportional to, the organic mass of the object. This assignment is justified because it is known that organic molecules, such as a variety of proteins and nucleic acids which have a similar refractive-index increment presumably because they have a somewhat comparable number and structural arrangement of C-, H-, O-, and N-atoms in a unit

mass, will produce about the same phase displacement of an unabsorbed light wave (Fig. I).

REFRACTIVE INDEX INCREMENT

$$\alpha = \frac{n_0 - n_m}{c}$$

PROTEINS	α	NUCLEIC ACIDS	α
Serum proteins	0.00179	Yeast nucleic acid	0.00168
Serum albumin	0.00181	Tobacco mosaic virus	0.00170
Serum globulin	0.00183	Desoxyribose nucleic acid	0.00190
Ox globin	0.00178	FATS AND CARBOHYDRATES	
Ox CO hemoglobin	0.00193	Fats	0.00140
β-lipoprotein	0.00170	Sucrose	0.00141
Egg albumin	0.00188	Starch	0.00131

FIG. I. Refractive-index increments (α) of proteins, nucleic acids, fats and carbohydrates. [See Adair and Robinson; Barer; Davies, and Wilkins.]

Thus, the organic mass is obtained from the interference pattern in an aqueous medium and is computed by the product of the area of the interference fringe and a numerical constant which depends upon the wavelength of the light employed. It is our practice now to use several monochromatic, unabsorbed wavelengths in the visible region and, occasionally, in the long-wave ultraviolet. The spectacular sensitivity of the method for the estimation of organic mass is shown in the last column of figures (Fig. II). For example, in the blue region of the spectrum at

Hg Line	λ (10^{-4} cm)	$\Delta = \frac{\lambda}{4}$ (10^{-4} cm)	$M_o = \frac{\Delta}{0.19} \times A$ (gm)	M_o (gm), for $A = 1\mu^2 = 10^{-8}$ cm^2
Y	0.578	0.144	$0.76 \times 10^{-4} \times A$	0.76×10^{-12}
G	0.546	0.136	$0.71 \times 10^{-4} \times A$	0.71×10^{-12}
B	0.436	0.109	$0.57 \times 10^{-4} \times A$	0.57×10^{-12}
V	0.405	0.101	$0.53 \times 10^{-4} \times A$	0.53×10^{-12}
UV	0.365	0.091	$0.48 \times 10^{-4} \times A$	0.48×10^{-12}

FIG. II. Organic mass, M_o, in gms. which produces a phase retardation, $\lambda/4$, in an interference microscope utilizing various monochromatic wavelengths of the mercury emission spectrum. In the last column the detectable organic mass in an area, A, of $1\mu^2$ is computed at various wavelengths.

wavelength, 0.436μ, the analyzable mass in 1μ^2 of are is 0.57 $\times$ 10^{-12} gm. Since our microscope will resolve about 0.1μ^2 of area, the minimum analyzable mass approaches one-tenth of these tabulated values, namely, about 0.6 $\times$ 10^{-13} gm., or 6 $\times$ 10^{-14} gm. at λ,0.436μ. As we shall show subsequently, this analyzable mass is much less than that of a single chromosome. It may well be, as estimated from several assumptions, that this is of the order of the mass of gene loci in giant chromosomes of invertebrate animals.

The accuracy of the interferometric method is evaluated by an analysis of the organic mass of the red blood cell in the normal and the pathological state (Fig. III). The average organic mass

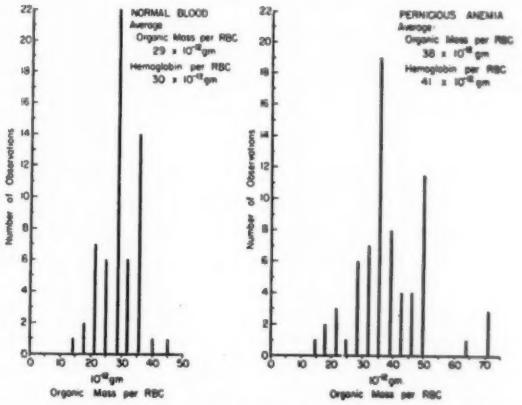

FIG. III. Analysis of individual red blood cells (RBC) by interference microscopy. The organic mass per RBC is plotted in the horizontal axis, and the frequency of occurrence of certain values is given in the vertical axis. Blood from a normal adult male is on the left and that from a patient with pernicious anemia is on the right. The average mass of hemoglobin per RBC is computed from determinations of the hemoglobin concentration and the RBC count.

of the normal red blood cell is found to be 29 $\times$ 10^{-12} gm. as determined by the interferometric analysis of individual red blood cells in a random sample. When the average quantity of hemoglobin per red blood cell is computed for the same specimen from the determination of the hemoglobin concentration and the red

blood cell count the value is 30×10^{-12} gm. Since 95–97 per cent of the organic mass of the red blood cell is hemoglobin, the agreement between the two analytical methods is excellent. An interferometric analysis of red blood cells in a specimen of blood from a patient with pernicious anemia yields an average organic mass of 38×10^{-12} gm. per red blood cell. The quantity of hemoglobin per red blood cell is 41×10^{-12} gm. as computed from the hemoglobin concentration and the red blood cell count.

Results

Thickness of a cell. The thickness of living mouse fibroblasts in tissue culture as determined by interferometry in air is plotted in this figure in relation to a position along an axial coordinate of the cell. The maximum thickness of these cells is about 4–5μ, and this value occurs near the geometrical center of the cell in accordance with expectation. The thickness decreases more precipitously along a semi-minor axis and more gradually along a semi-major axis.

Hydrous mass and organic mass of the cell. Average analytical data which are obtained by the interferometry of living mouse fibroblasts and sarcoma cells in tissue culture are summarized in these histograms (Fig. IV). The hydrous mass of the fibroblast

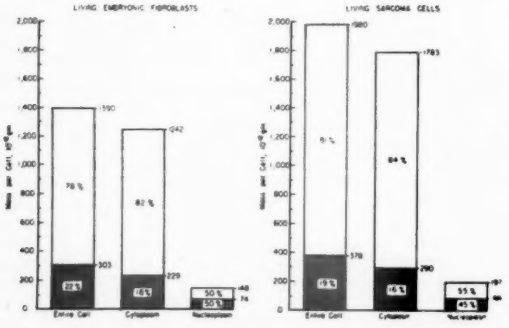

FIG. IV. Mass per cell as estimated by the interferometric analysis of living embryonic fibroblasts and sarcoma cells of the mouse in tissue culture. The mass is further partitioned between cytoplasm and nucleoplasm and between hydrous (no cross-hatch) and organic (cross-hatch) phases.

is 1390×10^{-12} gm. and 22 per cent of the total mass, or 303×10^{-12} gm., is organic mass. This partition of water and solids in the single cell is in good agreement with that generally reported for the gross analysis of a variety of mammalian tissues which, of course, include many different cellular and extracellular components. The data for the sarcoma cell disclose a hydrous mass of 1980×10^{-12} gm., and 19 per cent of the total mass, or 378×10^{-12} gm., is organic mass. Now, the total mass of the cell can be partitioned between the cytoplasm and the nucleoplasm, with less certainty in the latter case. When this is done about 90 per cent of the total mass is present in the cytoplasm and 10 per cent in the nucleoplasm. Furthermore, about 18 per cent of the total cytoplasmic mass is organic, whereas about 50 per cent of the total nuclear mass is organic mass. This is a remarkably high concentration of organic mass in the nucleus, and one may speculate, although I shall not, as to whether the solubility limits of some constituents are approached in the resting nucleus and perhaps temporarily exceeded in the dividing nucleus. The fibroblast and the sarcoma cell, it is to be noted, are quite similar with respect to the partition of mass between nucleus and cytoplasm and between hydrous and organic phases. The sarcoma cell differs, however, in being larger than the fibroblast in all respects considered.

Organic mass of sets of chromosomes. Recently, we have undertaken a multi-wavelength interferometric analysis of the organic mass of sets of chromosomes in dividing germinal cells of the mouse testis. This biological material is chosen for the evaluation of the analytical method because there is a geometrical progression in the number, and presumably in the mass, of chromosomes in the various maturation stages of the germinal cells. Thus, the fundamental or the haploid number, N, of chromosomes is present in the spermatid, the definitive germinal cell; the diploid number, 2N, occurs during the mitotic division of the secondary spermatocyte or in the reduction division of the primary spermatocyte; and the tetraploid number, 4N, is to be found during the mitotic division of some of the primitive germinal cells.

The study of chromosomes is not to be undertaken without recognition of the many fundamental contributions made by

investigators from The University of Texas, and notably those of Drs. Painter, Patterson, and Stone. A recent contribution which has been made from this University is that of Drs. Hsu and Pomerat who describe the advantages in the cytogolical preparation of chromosomes of placing cells in a hypotonic medium. Chromosomes so prepared have many features desired for interferometry: they are distributed over a greater area, tend to occupy one optical plane, and are more homogeneous, optically.

The interference photomicrographs of sets of chromosomes in germinal cells of the mouse testis are displayed on the top row of this illustration (Fig. V). Among the five examples there are

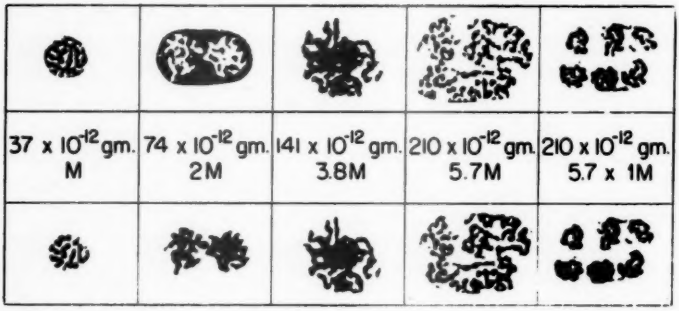

FIG. V. *Top row.* Photomicrographs of sets of chromosomes of germinal cells of the mouse testis taken with an interference microscope and monochromatic visible light. (λ, 0.436μ; 1000 X.) *Bottom row.* Facsimile presentations of the interference patterns obtained by an automatic optical density analyzer which also computes the area of the pattern. *Middle row.* Organic mass computed for the sets of chromosomes illustrated, which for the haploid number is 37×10^{-12} gm., designated as M.

a small array of chromosomes; a configuration that has separated into two parts; a large array; a very large group; and a cell containing six separate aggregations of chromosomes. You will recall that in the computation of organic mass it is necessary to know the area of the interference pattern. Now, the analysis of sets of chromosomes was undertaken with the knowledge that there existed in our Institute an optical density analyzer, designed for other work in accordance with specifications drawn up by Dr. Beyer of our Physics Department, that would automatically scan the interference pattern, print a facsimile of

what it saw, and compute the area by electronic counting circuits. The fascimiles of the interference patterns are displayed on the bottom row of the illustration, and they are essentially indistinguishable from the original interference patterns. The areas of these and of other interference patterns at different wavelengths and focal planes were analyzed automatically by the optical density analyzer; and the organic masses of the sets of chromosomes were computed from the product of the areas and the physical constants which have been previously discussed. The results are as follows. The organic mass of the small array of chromosomes is 37×10^{-12} gm.; that of the separating configuration is 74×10^{-12} gm., with an equivalent mass, 37×10^{-12} gm., for each part; and that of the large array is 141×10^{-12} gm. The organic mass of the largest array is 210×10^{-12} gm. A fortuitous circumstance occurred in the study of this cell for the large array of chromosomes was found to separate into six parts, each of which had an organic mass of about 35×10^{-12} gm. Now, if the organic mass of the small array of chromosomes on the left, namely, 37×10^{-12} gm. be designated as M, then the masses which have been observed are 2M, 3.8M and 5.7M. This is in remarkable agreement with the ratios, namely N, 2N, 4N and 6N for the haploid, diploid, tetraploid and hexaploid number of chromosomes which are provisionally assigned to these cells.

An interesting result is obtained when the organic mass of what is considered to be a diploid set of chromosomes is compared with the organic mass of a resting nucleus of a somatic cell, such as that of a fibroblast (Fig. VI). The average organic masses are identical. If the similarity of orders of magnitude is substantiated by further analyses, this would indicate that practically the entire organic mass of the resting somatic nucleus can be assigned to the diploid set of chromosomes contained within it. It has been thought by cytologists for many years that there is a direct relation betwen the size of the nucleus and the number of chromosomes, and this observation may eventually constitute proof of that belief.

The plans for further work are quite clear. Sufficient analyses of sets of chromosomes in germinal cells will be obtained so that the frequency distribution curves and coefficients of statistical variation may be obtained. A comparable study will be made of

chromosomes from the higher and lower orders of animals and from plants. A similar analysis will be performed on the dividing somatic cells. This should answer the question whether chromosomes of the somatic line of cells are as true to form as those from the germinal line. If, as some cytological evidence already indicates, the answer is in the negative, then the coefficients of variation of the organic mass of chromosomes in cells of chronically inflamed, hyperplastic, carcinogen-treated and neoplastic tissues will be evaluated. This should constitute a straight forward approach to the study of the most likely cause of the genesis of cancer, namely, the theory of somatic mutation. For if the organic mass of chromosomes displays wider variation, or increment or decrement, in precancerous or cancerous states as compared with the normal, then this is evidence of chromosomal, or possibly mutational, change.

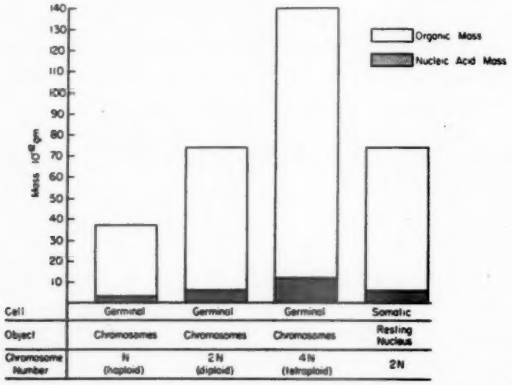

FIG. VI. Organic mass of haploid, diploid and tetraploid sets of chromosomes in germinal cells of the mouse testis as estimated by interferometric analysis; and the organic mass of the resting nucleus of a somatic (fibroblast) cell of the mouse.

Evidence in this respect is already being obtained from a different, although not unrelated, quantitative cytological analysis (Fig. VII). By means of ultraviolet microspectroscopy, the quantity of nucleic acid, a prominent component of the chromosomes, is determined in optically idealized (homogeneous) nuclei which

are isolated from somatic and neoplastic tissues of the mouse by a method taught to us by Mizen and Petermann. Somatic nuclei, such as those from the epithelium and the supporting connective tissue cells of skin, have a frequency distribution curve as shown on the upper left of this diagram. There is a unimodal distribution with a most frequent and average value of 6×10^{-12} gm. of nucleic acid per nucleus. A number of transplantable tumors, among them sarcoma Ma 387, sarcoma 180, carcinoma 1025, adenocarcinoma EO 771 and Ehrlich ascites tumor display a broader range of values; a most frequent value in the region of $12\text{–}16 \times 10^{-12}$ gm.; average values of 18, 16, 16, 16 and 13×10^{-12} gm., respectively; and in the case of sarcoma Ma 387, a suggestion of a bimodal distribution with peaks in the neighborhood of 12 and 24×10^{-12} gm. These observations are made on resting nuclei. If, as is likely, most of this nucleic acid can be

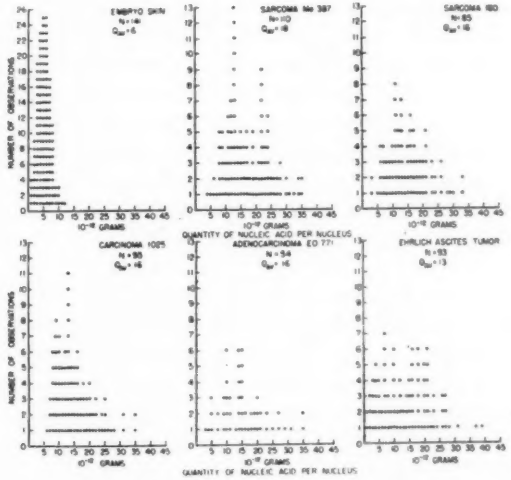

FIG. VII. The frequency of occurrence (ordinate) of various quantities of nucleic acid per nucleus (abscissa) expressed in 10^{-12} gm. for somatic and neoplastic cells of the mouse. The total number of observations is designated by N; and the average quantity of nucleic acid per nucleus is given by Q_{AV}. Data obtained by ultraviolet microabsorption analysis of optically homogeneous, isolated nuclei.

assigned to the chromosomes of the cell, then it follows that either the number or the composition of a set of these structures in the cancer cell departs markedly from that in a somatic cell. It may be found either that the ratio of the nucleic acid content to the organic mass of chromosomes is identical but that the total number of chromosomes differs or that the ratio of the nucleic acid content to the organic mass of the chromosomes is dissimilar in the two cells. There is evidence, at present incomplete, that both situations exist. In either case, and whether cause or effect, the theory of the mutagenic origin of the cancer cell can be put to test by the new analytical methods.

Aside from the foregoing, there are additional relations to be considered. The increased quantity of nucleic acid per nucleus in cancer cells of certain tumors of the mouse and of man, for example, in squamous carcinoma of the cervix, is a quantitative representation of the nuclear abnormality which comprises an important cytological criterion of malignancy. This is the increased stainability of the nucleus of the cancer cell with basic dyestuffs. It was upon this basis that we undertook the development of an automatic scanning microscope for diagnostic exfoliative cytology. For it was shown that if the cells of a smear were stained with a basic fluorochrome, that is, a fluorescing dyestuff, a population of cancer cells which desquamate from pre-invasive carcinoma of the cervix is discriminated, above and beyond the statistical laws of chance, from a population of normal cells by the greater nuclear light emission of the cancer cell.

Additional applications of the analytical methods of ultraviolet and interference microscopy include the study of the action of chemotherapeutic agents (Fig. VIII), such as triethylene melamine, or TEM, and of ionizing radiations on cancer cells. From the experimental work thus far the conclusion is emerging that, when the growth of a responsive tumor mass in the animal is arrested by these agents, the individual cancer cells continue to grow—in fact, they may become extremely large—but they do not divide. The quantitative data disclose that many of the cancer cells build up a sufficient quantity of nucleic acids for two sets of chromosomes, as though in preparation for division; and then these cells fail to divide and persist for various lengths of time with large or giant resting nuclei. Presumably the capacity for

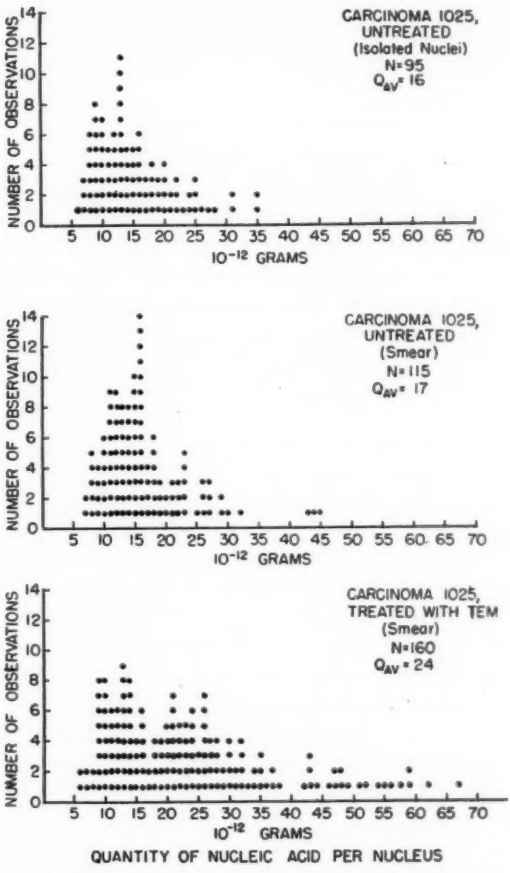

FIG. VIII. The frequency of occurrence (ordinate) of various quantities of nucleic acid per nucleus (abscissa) for neoplastic cells (Carcinoma 1025) of the mouse in untreated animals (top two graphs) and in those treated with triethylene melamine, TEM (lower graph), in a dosage which arrests tumor growth. The difference between the average values, Q_{AV}, for the untreated and the treated group is about six times the standard deviation of the difference between the means and is, therefore, statistically significant.

further division is lost because, according to Dr. Sugiura, tumor tissue composed of such altered cells when transplanted into a new animal does not survive.

Summary and Conclusions

The measurement of the retardation of the phase of a light wave is made with an interference microscope, and the retardation is assigned to, and used in the computation of, various structural properties of the cells, among which are the hydrous mass and the organic mass of the cytoplasm and of the nucleoplasm and the organic mass of sets of chromosomes. The application of the interferometric method to the study of the general physiology and pathology of the cell is presented, and an analytical approach to the theory of the mutagenic origin of the cancer cell is formulated.

By means of ultraviolet microspectroscopy the quantity of nucleic acid is determined in optically idealized (homogeneous) nuclei which are isolated from somatic and neoplastic tissues of the mouse. The nuclei of cancer cells in a number of transplantable tumors contain a quantity of nucleic acid which is two or more times greater than that in diploid somatic nuclei. When the growth of a responsive tumor mass in the animal is arrested by a chemotherapeutic agent, such as triethylene melamine, or by ionizing radiations, many individual cancer cells continue to grow and to build up a sufficient quantity of nucleic acids for two sets of chromosomes, as though in preparation for division, and then fail to divide and persist for various lengths of time with large or giant resting nuclei.

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SOME CYTOLOGICAL ASPECTS OF THE NUCLEIC ACID PROBLEM

THEOPHILUS S. PAINTER*

Biological workers have long regarded chromatin—or desoxyribonucleic acid, DNA for short—as one of the most important constituents of cells without which sustained life and reproduction are not possible. But for more than half a century our knowledge of chromatin was largely limited to morphological and genetic studies dealing with chromosomes and there was very little information about the chemical nature of this nucleic acid aside from its affinity for basic dyes. Within the past two decades, however, this situation has changed; biological chemists have greatly extended our knowledge of the nucleic acids—for there are two general types, desoxyribo- and ribonucleic acid—and what is especially important for cytologists and pathologists, microchemical techniques have been developed to a point where an experimental cytologist not only can identify both DNA and RNA but can measure with a fair degree of accuracy the amounts of these substances present in a single nucleus or cell cytoplasm. Among the more useful of these methods are: ultraviolet absorption spectra which permit the detection of nucleotides of both DNA and RNA in amounts which would not be visible by staining methods; a specific staining reaction for DNA (Feulgen's method) and photoelectric techniques which enable us to measure quantitatively the amount of stained material in different parts of a cell.

At the outset a few comments about nucleic acids seem desirable. Chromatin, or DNA, is normally confined to the nucleus. Cytologically two types are distinguishable. The one seems to be an integral part of the gene string, assuming that genes are nucleoprotein in character. The second type, called heterochromatin, is deposited on regions of chromosomes which are more or less genetically inert. Much evidence indicates

* From the Department of Zoology, The University of Texas, Austin. Presented at the Seventh Annual Cancer Symposium, May, 1953, at the M. D. Anderson Hospital for Cancer Research, Houston.

that heterochromatin is a source, possibly the principal one, of the nucleic acids of the cytoplasm. Ribose nucleic acid—formerly called yeast nucleic acid—is found mainly in the cytoplasm of a cell. Some occurs also in chromosomes and in the nucleolus. Chemically, DNA and RNA are both made up of nucleotides with certain differences in the sugar and pyrimidine components, but recent work indicates that these differences are not as great as once thought. The evidence suggests that DNA and RNA should be regarded as classes of substances rather than as specific chemical entities.

The first fact to be taken into account in this discussion is that RNA, or its nucleotides, are essential for protein synthesis, whether this synthesis results in a secretion of protein materials or in cell growth. Since many enzymes require co-enzymes containing ribonucleotides, this fact is not surprising but the quantity of RNA present is usually far beyond what would be required for enzyme systems and suggests other functions. An excellent example of a secretory cell is the royal jelly gland of the worker bee. This gland is normally functional from the sixth through the eleventh day of hive life. During this period, large amounts of RNA can be demonstrated in the cytoplasm of the gland cells, but when the worker bee turns to other duties, the cells remain intact but loses the RNA. An example of RNA in growth is the basophily of the cytoplasm of cancer cells so familiar to us all.

A second fact which seems to have widespread validity is that when cells are destined to undergo rapid proliferation, as in the cleavage of eggs, or require unusual amounts of RNA, as in the secretion of the royal jelly, a part of the required nucleic acid is provided from sources initially outside of the cell. That is to say, that while cells usually are able to synthesize the nucleic acids needed for normal growth, when there is to be a very rapid proliferation of cells, as in the cleavage of ova, or the production of secretions very rich in protein (royal jelly), there are demonstrable cytological mechanisms for supplying such cells with additional nucleic acids. I wish to present the evidence on which this conclusion is based because I think a similar mechanism may be functioning dur-

ing the rapid spread of neoplasms in advanced stages of certain types of cancer.

My own approach to a study of nucleic acids, from a cytological standpoint, in the middle thirties, stemmed from this question: If nucleic acids and nucleoproteins are very complex chemically, how is it possible for a fertilized egg to reduplicate the chromosomes some six or eight or more times during the course of twenty-four hours? As so often happens in science, the way a question is put sometimes points the way to its solution. Cytologists, of course, had long known that in ovaries of animals, there are two general methods by which the cytoplasm and yolk of the oocyte are formed; in one type this is done through the agency of so-called nurse cells with little or no participation by the nucleus of the egg. In the other type—eggs with germinal vesicles—the egg nucleus itself is very active in the elaboration of the yolk and cytoplasm. A careful study of these two types of eggs quickly showed the cytological origin of the needed nucleic acids.

The ordinary fruit fly, *Drosophila melanogaster*, has an excellent example of the nurse cell type of ovary. Initially, the egg follicle in the ovary consists of sixteen cells. The most distal cell is destined to become the definitive ovum. Its nucleus is quite small and achromatic throughout the formation of the oocyte. The remaining fifteen more proximal cells become nurse cells. Each such cell undergoes a series of endomitotic division cycles during which the chromosomes are reduplicated (Painter and Reindorp, 1939), and presumably the proteins in the cytoplasm as well, since the nurse cell grows in nuclear and cell size. In the end each of the nurse cells comes to contain a high number of sets of chromosomes (instead of two), usually 128 or 512 or more. Eventually, each nurse cell is bodily absorbed by the egg cell so that the mature oocyte contains the degradation products of thousands of chromosomes. While we do not know to what extent the DNA of the nurse cells is changed, it is obvious that there is available in the egg at the onset of development a very large amount of material derived from the chromosomes of the nurse cells. Presumably this accounts for the ability of cleavage nuclei to reassemble or synthesize new chromosomes so quickly.

In the toad egg, which was studied as an example of the germinal vesicle type of ovum, there is another type of cytological mechanism (Painter and Taylor, 1942), but the end result is the same; large quantities of nucleic acid derived from heterochromatic regions of the oocyte's chromosomes are stored in the cytoplasm of the toad's egg, during the months needed for the formation of mature eggs, so that when cleavage beings, as in the fruitfly, the degradation products of relatively large quantities of DNA are available to the chromosomes of cleavage nuclei.

Later studies (Painter, 1943) revealed in plants a mechanism quite comparable to the nurse cell mechanism of animals to supply the RNA needed for the rapid growth of pollen tubes in the mature pollen of Rhoeo. We need note here only the fact that the tapetal cells, which on autolysis furnish the source of the RNA, undergo endomitosis prior to their dissolution, thereby elaborating considerable quantities of chromatin which presumably is the source of the RNA.

Two more bits of information should be mentioned. Through a series of studies, using ordinary chemical methods, Brachet, (1933 and later) was able to demonstrate that prior to fertilization the sea urchin egg contains relatively large quantities of RNA and only a very small amount of DNA. As cleavage goes forward, the amount of RNA decreases while that of DNA goes up. From this we may conclude that nucleic acids exist as such in the cytoplasm of eggs and that somehow the RNA is the source of the DNA of cleavage nuclei. Another bit of evidence may well be included here. In insects with complete metamorphosis, *i.e.*, with larval-pupal-imago stages, growth during the larval stages is largely by endomitosis. There are, for example, about the same number of cells in the salivary gland or in a given part of an intestine in a larva say 2 mm. in length as in a larva 2 cm. in length, but the cells and their nuclei are very much larger in the latter. With the onset of the pupal stage, such larval organs undergo autolysis and form the nutritive mass, or brei, from which the imaginal discs, or anlage, of the adult organs derive their nourishment. One is tempted on the basis of such evidence to generalize and say that the endomitotic type of growth of cells typically obtains

when such cells are destined to be used as a source of nucleic acid for other cells.

From this background of information I now turn to neoplasms and raise the question: Where do the necessary nucleic acids come from when, as commonly occurs in advanced stages of many cancers, there is an almost explosive growth or proliferation of malignant cells? As a cytologist, and on the basis of what is known about such rapid growth and proliferation in developing ova, for example, and the other facts and consideration which I have presented, I would expect that such rapidly dividing cells are obtaining some nucleic acid, or its precursors, from an exogenous source. The alternative explanation would be that in old cancers there was a great increase in the ability of cancer cells to synthesize their own nucleic acid. In view of all the facts, it seems to me that the first explanation is the more likely one.

As you are well aware, a cytological examination of a large cancer shows two general types of cells. The first may be called normal cancer cells which divide mitotically and give rise to other cancer cells. The second type of cell is characterized by greatly enlarged hyperchromatic nuclei. Such cells do not divide normally, if at all, and their ultimate fate is autolysis. One of my students, Dr. Biesele, measured the nuclei of this second type and on the basis of nuclear volumes, as well as sporadic divisions when high chromosome numbers are observed, concluded that the chromosome had been reduplicated a number of times (Biesele, Poyner, and Painter, 1942). Thus this second type of cancer cell is quite similar cytologically to typical nurse cells, as these are found in the ovarian follicles of the fruit fly. What becomes of the nucleic acids of these large cancer cells when they undergo autolysis? To a cytologist the answer suggested is that it becomes available for the use of other growing cells, that is, as a nucleic acid source for normal cancer cells.

If this suggested explanation has validity then one might envisage the growth of a cancer in the following terms: In early stages cancer cells synthesize their nucleic acid from precursors of the blood stream and in competition with other cells of the organism which require nucleic acid precursors for

their normal functioning, such as reduplication or secretion. So the neoplasm grows slowly at first forming many cells which are incapable of normal cell division but which store up large quantities of DNA. Eventually, the autolysis of these atypical cancer cells releases the stored nucleic acids and probably other substances needed for growth and the normal cancer cells proliferate very rapidly.

Although I have not seen the above explanation in any of the articles I have read, it would be surprising if some others have not had the same thought. There is some direct evidence to support this viewpoint in some recent experiments reported to Dr. Biesele by Dr. Hans Lettré, a renowned investigator in the general field of mitotic poisons. As told to me, Dr. Lettré has injected mitochondria into mice carrying an ascites cancer. When the source of the mitochondria was from cancer cells, there was a marked increase in the proliferation of the cancer cells after a period of some ten hours. When the mitochondria injected were from normal tissue, no such increase in division was observed.

In conclusion I may state that we have under way, in Austin, experiments which we hope will give decisive evidence as to the validity of the explanation advanced for the extremely rapid growth of cancer cells in late stages of some malignancies.

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SMOKING AND CANCER OF THE LUNG—LET'S REVIEW THE FACTS

R. H. Rigdon and Helen Kirchoff*

The use of tobacco is very ancient. In this country there is a legend that during a severe famine a heaven-sent maiden descended and caused Indian corn, potatoes, and tobacco to spring up (Rolleston, 1926). Columbus, in 1492, was impressed with the Indian chiefs who puffed rolls of tobacco leaves. It was first introduced into England between 1565 and 1585 (Rolleston, 1926). During the subsequent 100 years both the merits and harmful effects of smoking were discussed frequently. In 1707 apparently the first "tobacco controversy" occurred in England—"half of the town was on one side, and half on the other" (Editorial, *The Lancet*, 1857).

One hundred and fifty years later a second controversy arose and the pros and cons were published in *The Lancet* during the spring of 1857. At that time it was said that tobacco acts on the mind by producing inability to think, loss of memory, hysteria, insanity, and an irritable temper. It acts on the respiratory organs by causing consumption, hemoptysis and an inflammatory condition of the mucous membrane of the larynx, trachea, and bronchi, a short irritable cough and hurried breathing. "It impairs the vigour and energy of the English people, and causes them to sink in the scale of nations; it has caused the governmental evils of Turkey; it ruins young men . . . and renders the old women in Ireland troublesome to the dispensary doctors." Others, of course, contended that smoking does not cause insanity for more women are insane than men; that, on the contrary, it is a useful sedative in cases of insanity (Editorial, *The Lancet*, 1857).

Statistics on the frequency of smoking apparently were not obtained at the time of the earlier controversy. In *The Lancet* of 1857 (Editorial) it was said that "the use of tobacco is

* From the Laboratory of Experimental Pathology, The University of Texas Medical Branch, Galveston, Texas. Presented at the Seventh Annual Cancer Symposium, May, 1953, at the M. D. Anderson Hospital for Cancer Research, Houston.

widely spread, more widely than any one custom, form of worship, or religious belief, and that therefore it must have some good or at least pleasurable effects; that, if its evil effects were so dreadful as stated the human race would have ceased to exist." A student at Cambridge expressed the opinion that "by far the greater half of the undergraduates here smoke" (Editorial, *The Lancet*, 1857). In a smoking survey made in June, 1952, at the Medical Branch of The University of Texas, it was found that 62 per cent of the students smoked. In 1884 Blodgett said of the boys in England, "Nearly all boys now smoke cigarettes to an inordinate extent." Sir Humphrey Rolleston wrote in 1926 that "considering the universality of the habit and the large number of heavy smokers, the comparative rarity of undoubted lesions due to smoking is remarkable." In that paper Rolleston did not refer to smoking as a cause of cancer of the lung.

Tobacco was used in the earlier periods in cigars, pipes, and for chewing and the dipping of snuff. The word cigarette according to Garrison (1897) was first used as an English word in 1842. Two years later it was stated that "the cigarette has but little strength, it can do you no harm" (Garrison, 1897). Cigarette smoking was not popular in England until 1870. They were first manufactured in America about 1864; however, they were not made by machines until 1910 (Haskin, 1926). The habit of cigarette smoking has progressively increased since that time as indicated by the following figures supplied by the U. S. Department of Agriculture:

Per Capita Consumption of Tax-Paid Tobacco Products in the U.S.

Calendar Year	Cigarettes Number	Cigars Number	Smoking Tobacco-Lbs.
1900.....	32	73	1.07
1910.....	94	77	1.76
1920.....	419	80	1.36
1930.....	972	50	1.32
1940.....	1369	42	1.54
1950.....	2346	36	.59

It is impossible to satisfactorily estimate the per capita consumption of tax-paid cigarettes within the United States because many states have been taxing cigarettes for only a few

years. Large quantities of tax-exempt cigarettes have been shipped from one state to another, presenting another problem referable to consumption within the state. Furthermore, some states have more efficient tax-enforcement laws than others.

Little data are now available on the frequency of smoking. This factor alone is essential in any study of smoking referable to cancer of the lung. During the eighteenth and nineteenth centuries both men and women smoked. The cigarette habit became popular with women in the United States about 1920. It was pointed out by the Metropolitan Life Insurance Company in 1939 that the smoking habit since 1917 has become relatively more prevalent among women than among men. In a survey made in 1935 *Fortune* Magazine found that 37 per cent of the persons interviewed smoked cigarettes, 65.5 per cent of the men under 40 and 39.7 per cent over 40 years of age smoked, 26.2 per cent of the women under 40 and 9.3 per cent over 40 years of age smoked. In November of 1950 *Collier's* Magazine (Maisel) estimated that "more than three-fourths of all adult men and more than 40 per cent of all women smoke." In 1947 Mills and Porter (1950) interviewed 310 white women, 20 to 29 years of age, in Columbus, Ohio, and found that 39.68 per cent smoked cigarettes. Of 520 white women, of similar age, interviewed in Cincinnati, Ohio, during the same year by Mills and Porter (1950), 41.35 per cent smoked cigarettes. Of 1,153 white men, 20 years of age and older, interviewed in 1947 by the same investigators (1950), 41.7 per cent smoked cigarettes, 25.6 per cent smoked a pipe, cigars or a combination of these, while only 32.6 per cent were nonsmokers. Mills and Porter (1953) say it is imperative that a base-line of smoking habits be established in respect to age, sex, and race of standard groups of population and emphasize that "well people at their homes, rather than hospitalized or ambulant clinic patients, should be utilized in studies of the relationship of tobacco smoke and disease."

One of the most important factors to be established today is the frequency of cigarette smoking in the population. As you know, there are several statistical studies involving select groups of hospital patients with and without cancer of the lung. Data are essentially nil, however, referable to smoking and other

pathological processes. Such a study has been in progress in our laboratory for a year. The data so far have been obtained from the following sources:

Medical Branch personnel	1,000
College and professional students	5,900
Patients at John Sealy Hospital	3,100
Total	10,000

We do not wish to imply that the above sample is representative of the population. It is of interest to note the number of males and females that smoke in this group of 10,000 persons[1] (Table 1). Figure I shows the percentage of individuals in the

TABLE 1

Shows the present, past and non-smokers in 10,000 individuals according to sex, race and age

Sex & Race	Age in Years 15–19	20–24	25–29	30–39	40–49	50–59	60–69	70–79	80+	Total
					Present Smokers					
WM	420	740	425	237	164	138	114	60	9	2,307
WF	158	189	121	134	109	40	15	9	0	775
CM	210	172	51	61	63	47	52	32	9	697
CF	193	208	86	95	57	23	14	2	0	678
					Past Smokers					
WM	74	68	59	35	19	21	24	22	6	328
WF	22	9	8	13	14	7	2	1	0	76
CM	19	13	4	5	3	8	17	8	0	77
CF	15	8	8	8	7	5	8	1	0	60
					Non-Smokers					
WM	694	508	147	58	30	23	31	15	7	1,513
WF	703	229	100	144	161	109	93	54	10	1,603
CM	212	110	16	14	21	23	12	7	4	419
CF	639	288	105	136	123	89	54	30	3	1,467
TOTAL	3,359	2,542	1,130	940	771	533	436	241	48	10,000

different age groups that either now smoke or have smoked in the group of 10,000. The similarity of smoking habits in the white and colored male and the white and colored female in the different age groups is interesting. From these data approxi-

[1] Data from a study to be published in cooperation with Dr. Harvey Haag, Medical College of Virginia.

mately 10 per cent of individuals discontinue smoking once they have begun.

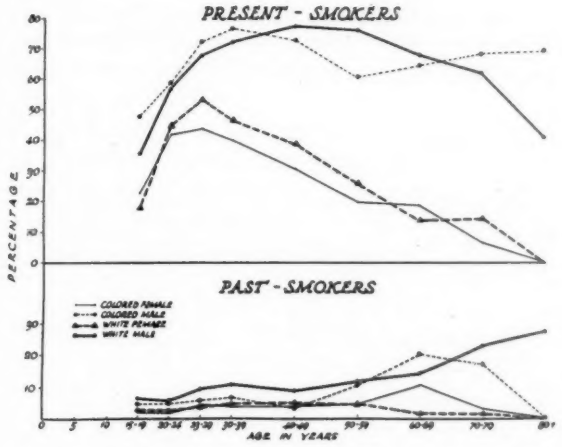

FIG. I. Graph showing the percentage of individuals in the different age groups that either now smoke or have smoked in the group of 10,000 individuals.

Pathologists appreciate the fact that the problems in the diagnosis and the attempt to determine the frequency of cancer of the lung have been equally as difficult to establish as that of determining those who smoke, and the amount and length of time they have smoked. May we remind you that an undifferentiated carcinoma of the lung was considered for many years to be a sarcoma of the mediastinum? Our vital statistics include many such cases. Furthermore, any attempt to determine the frequency of bronchogenic carcinoma in relation to other neoplasms is unsatisfactory since our concepts of what should and should not be included as a malignant tumor have varied for classification purposes. Death certificates give an adequate sample for a statistical study. However, the authenticity of many of these may be questioned. The U. S. Public Health Service in 1950 (Cancer Mortality in the U. S.) observed from their study of death certificates that the diagnosis of cancer is more reliable than the site or origin of the neoplasm.

In 1950 we had the opportunity to compare the cause of death given in the postmortem protocol with that recorded on the death certificate (Rigdon and Kirchoff, 1951). The degree of correlation was low. To cite one interesting case, a neurosurgeon gave the cause of death as "edema of the brain" when his patient died within 24 hours after the removal of a brain tumor.

One may disagree with the advice given by Dr. Weller (1929) relative to the diagnosis of cancer of the lung. He said, "Only those reputed examples of primary carcinoma of the lung are of value on which a complete autopsy has been done. . . . There must be microscopic verification of each case, if the results are to be fully accepted. . . . Every experienced prosector can recall autopsies in which painstaking search failed to disclose a primary site of malignant growth although abundant secondaries were present in the lungs or in the liver. . . . Cases are encountered too, in regard to which positive conclusions cannot be drawn even with thorough microscopic study. . . . In studies which are only statistical, the reader is at a loss to know how critical the compiler may have been in selecting his original units."

The techniques for the diagnosis of cancer of the lung certainly have improved during the past few years. However, some of our statistics were compiled when such techniques were unknown. Biopsies and cytological examinations contribute to better diagnoses. We would like to cite you a case which clinically and from the biopsy all of us considered to be a bronchogenic carcinoma. At autopsy, the patient had cirrhosis of the liver with a primary hepatic cell carcinoma that had metastasized to the lung and had invaded the wall of a medium-sized bronchus near the hilus.

Recently at a clinical pathlogical conference a clinician presented a case of a primary cancer of the lung and emphasized the fact that the patient had smoked two packs of cigarettes a day for 20 years. The tumor turned out to be Hodgkin's disease. We hope that you will pardon us for calling to your attention these cases, the like of which all of you have encountered. We wish only to emphasize the fact that records of similar cases

are frequently the basis for statistical studies determining the relation of cigarette smoking to cancer of the lung.

Kreyberg at the University of Oslo wrote in the *British Journal of Cancer* in June of 1952 that "up to the present time the general mortality statistics are so unprecise that they must be used with great reservation as a base for the study of the actual occurrence of lung cancer."

We are sure that all of you are seeing more cases of bronchogenic carcinoma today than ever before. No doubt, you are also seeing more cases of all cancer than you have previously. In data gathered in Berlin, Hanf (1927) found that, between 1903 and 1906, 7.9 per cent of the cases examined at autopsy had a cancer, of which 3.3 per cent were lung cancers. Between 1922 and 1925, the corresponding figures were 17.7 per cent and 7.5 per cent. A 50 per cent increase in cancer with a corresponding increase in the percentage of cases of cancer of the lung, therefore, occurred within a period of twenty years. Koletsky in 1938 in Cleveland, Ohio, found 13.8 per cent malignancies in 7,685 autopsies; 9.39 per cent of these were primary in the lung. Rosahn in 1940 in Connecticut found 10.1 per cent of all neoplasms observed at autopsy to be primary in the lung. Recent necropsy reports from veterans hospitals have shown a very high percentage of cases of cancer of the lung. Tanner *et al.* (1952), reported 33.6 per cent of their autopsy cases had a malignancy and 20.6 per cent of these were primary in the lung. If we accept such data, based upon a selected group in which the percentage of postmortem examinations is very high, and compare it with the vital statistics of yesteryears, without any adjustments, our opinion of the frequency of cancer of the lung will be greatly distorted.

Clinical interest in cancer of the lung, the stimulated interest in all cancer through the public press, mass x-rays for tuberculosis and new methods in diagnosis have contributed to the increased frequency of cancer of the lung. Today, more than ever before, the use of antibiotics is contributing to this increase because many people between the ages of forty and sixty who previously died of pneumonia are now being successfully treated for this infection. It frequently happens that the basis for the pneumonia is a small cancer of the lung.

The U. S. Public Health Service published between 1950 and 1952 a study of the incidence rate for cancer of the bronchus and lung per 100,000 population in ten cities for the years 1938 and 1948. Their findings are as follows:

City	1938	1948	Increase
Atlanta	2.9	8.9	3.06
Dallas	3.6	16.3	4.52
Birmingham	4.7	13.6	2.89
Denver	6.6	14.8	2.24
New Orleans	7.6	20.8	2.60
Detroit	7.6	19.0	2.50
Pittsburgh	8.1	15.7	1.93
Chicago	9.1	15.9	1.74
Philadelphia	9.1	18.3	2.01
San Francisco	9.8	20.8	2.12

(Cancer Morbidity Series 1-10 P.H.S. 1950–52)

It may be difficult to account for these variations in the frequency of cancer of the lung in specific cities only on the basis of the smoking of cigarettes. Note that the increase is greatest in Dallas. Can this be related to the fact that "the biggest always occurs in Texas"?

Joannovic in 1923 was one of the first to list smoking as a cause of cancer of the lung. Only a few publications have questioned this relationship. Table 2 gives the data from three different stud-

TABLE 2

Presents data from 3 studies by other investigators, giving the number and percentage of cigarettes smoked by patients with and without cancer of the lung

	Total No Cases	Number and Percent of Cigarettes Smoked									
		1	%	5	%	15	%	25	%	50+	%
Exp. A											
Cancer	647-M	33	5.1	250	38.6	196	30.3	136	21.0	32	5.0
Control	622-M	55	8.8	293	47.1	190	30.5	71	11.4	13	2.1
		0	%	0-10	%	10-20	%	20-40	%	40+	%	Unk.	%
Exp. B													
Cancer	144	13	9.0	5	3.5	28	19.5	78	50.7	22	15.3	3	2.1
Control	144	44	30.5*	16	11.1*	27	18.8	50	34.8	5	3.5	2	1.4
						<10	%	10-20	%	>20	%		
Exp. C													
Cancer	68					6	8.8	32	47.1	30	44.1		
Control	138					30	21.7*	76	55.1*	32	23.2		

Experiment A - Doll and Hill, 1950; B - Breslow, 1951; C - McConnell et al. 1952

* These controls show a higher percentage of smoking than the cancer group. From these data, we may conclude that smoking 15 or less cigarettes a day will protect one against cancer of the lung.

ies showing the number of cigarettes smoked by individuals who had cancer of the lung. Regardless of the fact that some of the groups have very few cases, we would like to emphasize that in each of these three studies the percentage of individuals who smoked less than 15 cigarettes a day and did not have cancer of the lung is much higher than that of the corresponding group who had bronchogenic carcinoma. Furthermore, as far as we know the cases of cancer of the lung studied by Doll and Hill (1950) and Breslow (1951) were not proven by pathologic examination, while the study by McConnell and associates (1952) was based on proven cases of cancer of the lung. The latter investigators concluded that "no significant difference was found in the incidence of smoking, or of the proportion of cigarette-smokers, in the two groups." Sadowsky *et al.* (1953) have demonstrated an association between cigarette smoking and cancer of the lung, between cigarette smoking and laryngeal cancer, and a suggested association between cigarette smoking and cancer of the esophagus. However, they conclude "aside from the statistical significance and apparent realness of these associations there remains the question of whether smoking is etiologically related to lung cancer."

Wynder and Graham stated in 1950 that "the greater practice of inhalation among cigarette smokers is believed to be a factor in the increased incidence of the disease." Doll and Hill (1950) in a similar statistical study state that "it would appear that lung-carcinoma patients inhale slightly less often than other patients." In another statistical study, Schrek *et al.* (1950) concluded "the relative low percentage of deaths by cancer of the respiratory tract indicates that smoking is, at most, only a weak carcinogenic agent."

As you know, Dr. Ochsner has been interested in smoking and cancer of the lung for several years. However, from a review of his publications it would seem that his opinion has varied relative to this relationship. In 1941 he presented a graph showing the comparison of the death rate per 100,000 population from cancer of the lung with the *production* of tobacco and automobiles in the United States during the period 1920 to 1936. Of automobiles and cancer of the lung, Ochsner and associates concluded that there was no significant relationship, but they did find an obvious par-

allelism between the increased *production* of tobacco and cancer of the lung. In 1948 Ochsner and his group stated that "although we previously were of the opinion that the chronic irritation resulting from excessive cigarette smoking was a factor, this cannot be proved. However, the fact that there is a parallelism between the number of cigarettes sold in the United States and the increased incidence of bronchiogenic carcinoma is interesting. . . . In our series we have not been able to show that there has been a higher incidence of smokers than in the average population of a whole." Then in 1952 Ochsner said, "For some time two of use have been convinced that there is a causal relationship between the increased incidence of bronchiogenic carcinoma and the increased use of cigarettes." Ochsner (1951) said that Dr. Evarts Graham has said to him, "Yes, there is a parallelism between the increased incidence of bronchiogenic carcinoma and the sale of cigarettes; but also there is a parallelism between the increased incidence of bronchiogenic carcinoma and the sale of nylon stockings." This smoking situation and cancer of the lung reminds us of a recent cartoon in *The Saturday Evening Post* which stated, "The bureau is unable to tell us how come the 4 per cent increase in marriages in Arkansas in 1951 is also matched by the increase in the sale of shotgun shells."

Doll and Hill (1952), well known statisticians in Great Britain, say, "It would seem that the recorded differences in mortality between town and country are greater than could be attributed wholly to the differences in smoking habits. In other words, the differences in smoking habits are not sufficient to lead to a rural mortality rate which is only about half that of the large towns." Furthermore, the same investigators state that "the increase in the number of deaths recorded is relatively greater than the increase in the consumption of tobacco." We (Rigdon and Kirchoff, 1952) have recently found "a significant correlation in the United States between the population to physician ratio and the population to bed ratio and the number of reported deaths from cancer of the lung. Better diagnoses, decrease in the number of deaths from infectious diseases by both prevention and therapy, and the increase aging of the population seem to be more significant factors than any one or group of carcinogens to account for the apparent increase in the frequency of carcinoma of the lung.

In November, 1952, an experimental study was reported in *Science* (Essenberg) showing that mice exposed to cigarette smoke developed cancer of the lung. A similar study by Lorenz (1943) did not show the same results. The basis for this variation needs to be studied. If a carcinogenic agent can be shown to be the cause of the increase in cancer of the lung and if this agent occurs in tobacco smoke, the industry then should exert every effort to remove it. Until this relationship is proved, scientists should pursue the recommendations made at the Symposium on the Endemiology of Cancer of the Lung held in Belgium in 1952. These recommendations state, "The smoking of tobacco —especially cigarettes—has often been regarded as a causal factor of cancer of the lung. . . . Further research on this subject is imperative." The Chocorua Lung Cancer Conference held in September, 1952, in New Hampshire concluded that "work should be continued to determine whether or not smoking is a major cause of lung cancer."

In summary it may be said that in our opinion the data available today do not justify the conclusions that the increase in the frequency of cancer of the lung is the result of cigarette smoking. The statement that carcinoma of the bronchus has actually increased in frequency is in our opinion open to question. Furthermore, adequate studies are not now available on the frequency of cigarette smoking in the population. These factors would be necessary in establishing a causal relationship between smoking and cancer of the lung.

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THE ELECTRON MICROSCOPY OF NORMAL AND NEOPLASTIC CELLS

CECILY CANNAN SELBY*

Electron microscopy occupies a unique position among the various microscopic techniques available today as the only one which permits direct visualization of structure in the 10–100 mμ size range. Since larger structures may also be observed, electron microscopy has already contributed to cellular research in at least two directions; microscopic structures have become better understood by observation at higher resolving power, and previously invisible submicroscopic structures have been revealed. This high resolving power is achieved simply through using electrons with associated wavelengths of the order of 0.05 A as illumination. Because the electromagnetic lenses which must be used for focusing the electron beam cannot be corrected for spherical and chromatic aberrations, resolutions of 8–10 A are the experimental limit of the electron microscope despite the much shorter wavelength of the electrons.

Although the electron microscope is, in theory, rather an exact counterpart of the light microscope it imposes quite different requirements for specimen preparation. All specimens must be thoroughly dehydrated to be placed in the high vacuum under which the instrument is operated, so that there is, as yet, no possibility of observing wet or living specimens. Because image contrast in the electron-optical image arises primarily from variations in atomic densities, adequate atomic density contrast must be present or induced in the specimen. Most materials of biological interest consist predominantly of the elements C, N, and O which have nearly the same atomic weight, so that contrast arises mainly from variations in local concentrations of these elements, or may be induced by staining or fixing with such electron-dense compounds as osmium tetroxide, phosphotungstic acid or phosphomolybdic acid. The

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most serious specimen requirement is that, to avoid excess electron absorption, specimens must be no thicker than .1–.2 μ for resolutions less than .1 μ and even thinner for higher resolutions. During the past first decade of application of the electron microscope to biological problems, therefore, much time has had to be spent in developing methods for preparing tissues sufficiently dry, sufficiently thin and with sufficient contrast for observation in this new instrument. The methods achieving this which are now employed for tissue studies involve isolating cell components, making replicas of the surface of thick specimens, selecting thinly spread portions of cultured or smeared cells or sectioning embedded whole tissue. Only the latter two methods allow observation of portions of intact cells and are therefore those with which we have been mainly concerned.

Since thin-sectioning has become practicable only within the past year or two, our studies began with cells in tissue culture prepared for electron microscopy by the method introduced by Porter, Claude, and Fullam (1945). Cultures are planted upon a plastic film and, after fixation in drops or vapor of the desired fixative, transferred together with the film to which they adhere to the supporting screen of the electron microscope. Portions of well-spread cells can then be found which are sufficiently transparent to the electron beam. Following the original authors, fixation in osmium tetroxide vapors overnight was used throughout. Osmium fixed cells have been shown, by a variety of criteria, to approximate the living cell more closely than cells fixed by any other method (see the review of Selby, 1953). Their electron-optical image also shows excellent contrast. The chemical action of osmium tetroxide (commonly known as osmic acid) is therefore of great interest and has recently been reviewed by Porter and Kallman (1953). This fixative is known to combine rapidly with unsaturated fatty acids to produce unstable osmium esters which decompose to deposit osmium oxides or hydroxides at the site of activity. Slower oxidation processes (at aromatic double bonds) may then follow. Very little is known of its reaction with proteins, but Porter and Kallman (1953) show that it quickly causes proteins to gel (with pH shift from 7.4 to 6.4) without the coarse coagulation images present in the gels produced by other

fixing agents. They suggest that it produces an initial partial denaturation of proteins with a concurrent gelation which renders many components of the cell insoluble, and that this is followed by a further denaturation and hydrolysis of certain proteins which produces soluble end-products capable of being washed out of the cell.

In the electron-optical image of an osmium fixed cell therefore, the lipid inclusions are the most electron-dense while the density of other formed elements is roughly proportional to their content of protein and lipoprotein. It has unfortunately not yet been possible to determine a simple relation between the mass density of an object point in the specimen and the intensity in the corresponding image, so that quantitative measurements of cellular densities cannot be made from an electron microscope image.

In well-spread cultured cells, following fixation for only 5 to 10 minutes, mitochondria and lipid inclusions are clearly defined but submicroscopic structures are largely obscured by an amorphous and frequently fibrous matrix which is removed following further fixation and extensive washing. The formed components which then appear are listed in table 1. The lipid inclusions, mitochondria and endoplasmic reticulum appear in all cells and are illustrated in figures 1 and 2 and 3. The mitochondria which flattened down (*i.e.*, dehydrate) considerably

TABLE 1

CYTOPLASMIC COMPONENTS

Tissue Culture	Tissue Sections	Size Range (mμ)
lipid inclusions		
mitochondria		150-700
ultra-mitochondria		100-150
thread-like granules(?)	"double membranes"	50-70
"endoplasmic reticulum"	microvesicles	50-150
filaments		20-100
"virus-like" particulates (uniform morphology)		40-400

during drying show evidence of an enclosing membrane but do not reveal any clear internal structure because of drying artefacts and confusion with over- and under-lying material. The "endoplasmic reticulum" is the name given by Porter and Kallman (1952) to the lightly osmiophilic elements which may appear in a variety of forms varying from vesicular to canalicular depending upon the condition of the cell at fixation.

Other submicroscopic particulates, with the same degree of osmiophilia as mitochondria but of a smaller size, were first observed in cells of a rat sarcoma by Porter and Thompson (1947). They have subsequently been found by us (Cannan (Selby) and Berger, 1951; Selby and Berger, 1952) to predominate in all human and mouse neoplastic cells cultured, and by a group of French workers (Oberling, Bernhard, Braunsteiner, and Febvre, 1950; Oberling, Bernhard, Febvre, and Harel, 1951) to be common in leukemic white cells. Until their function is better understood, we prefer to call these particulates simple "ultra-mitochondria" (following Oberling's 1951, term "ultra-chondriome"). They generally appear morphologically different from mitochondria in that they do not flatten down as much during drying and frequently adopt a chain-like configuration uncommon in mitochondria. Histograms constructed from measurements of the smallest diameters of all cytoplasmic particles in normal, embryonic and neoplastic human epithelial cells indicated that these ultra-mitochondria also possess a size distribution independent of that of the normal mitochondria (Selby and Berger, 1952). Examples of their morphology are given in figures 2 and 3.

Following the initial observation of ultra-mitochondria it was reported (Porter and Kallman, 1952) that the same particles could be very occasionally observed in normal adult fibroblasts and more frequently in embryonic rat fibroblasts. They were also reported to be present in a certain percentage of normal leukocytes (Oberling, Bernhard, Febvre, and Harel, 1951). However, in extensive studies of normal and neoplastic human epithelial cells (Selby and Berger, 1952) and mouse epithelial and fibroblast cells (Cannan (Selby) and Berger, 1951) and analysis of 2,000 electron micrographs we were unable to find particles, distinguishable from small mitochondria in size or quantity, in

the normal cells while typical ultra-mitochondria appeared in masses in every tumor cell examined. In two instances it was possible to compare cells grown from adjacent normal and neoplastic sites of the same patient (see Figs. 1 and 2) and include the primary tumor and metastases. To compare the tumor cells with cells of equal proliferative rate, cells were cultured from several mouse embryos and from a 12-week human embryo. These cells showed a growth rate and clot lysis in culture similar to that of neoplastic cells, but their cytoplasm was indistinguishable from that of adult normal cells and ultra-mitochondria failed to appear.

In only about 1 per cent of the normal adult or embryonic epithelial cells examined, certain threadlike granules were observed, similar to ultra-mitochondria in osmiophilia but with a relatively constant diameter of 70 mμ (the ultra-mitochondria has an average diameter of about 120 mμ) and a uniformly threadlike morphology. They therefore appear different both in size and morphology from the granules in tumor cells, as illustrated by comparison of figures 1 and 2. Their very rare appearance may be due either to their failure to migrate to the cell periphery, their absence in cells spreading well at the periphery of a culture, or their identity as some contaminant in the preparation.

The other cytoplasmic components noted in table 1 are observed only in specific cell types. Submicroscopic filaments have been noted in briefly fixed cultured fibroblasts (Bang and Gey, 1948) and in cultured embryonic nerve cells (deRobertis and Sotelo, 1952). Clusters of submicroscopic particles of very uni-

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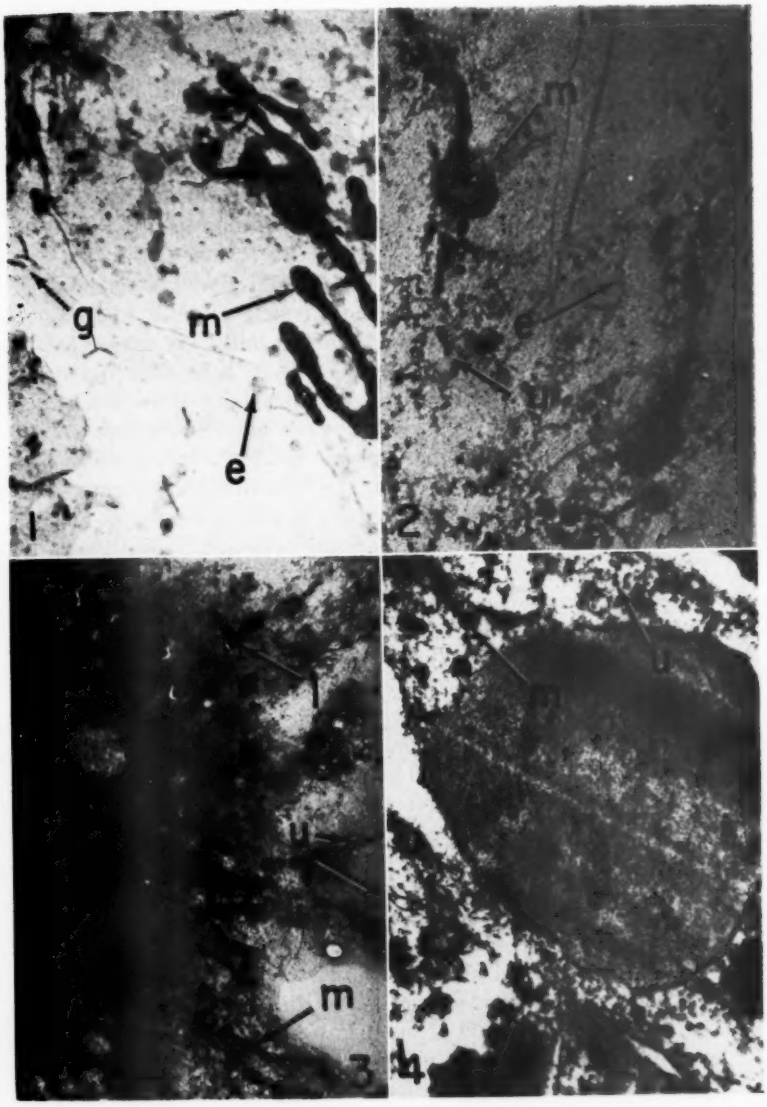
PLATE I

FIG. 1. Electron micrograph of the periphery of an epithelial-type cell in a culture from a specimen of normal human buccal mucosa. Mitochondria (m), endoplasmic reticulum (e), and narrow thread-like granules (g) are illustrated. Magnification, X 10,000.

FIG. 2. Electron micrograph of the periphery of a malignant epithelial-type cell in a culture of a servical lymph node containing metastases of a squamous cell carcinoma of the mouth of the same patient from whom the normal specimen of figure 1 was obtained. Mitochondria (m), ultra-mitochondria (u), and endoplasmic reticulum (e) are illustrated. Magnification, X 10,000.

FIG. 3. Electron micrograph of the periphery of a cell cultured from the 98/15 mouse hepatoma. Lipid inclusions (l), ultra-mitochondria (u) and mitochondria (m) are illustrated. Magnification, X 4,500.

FIG. 4. Thin section of a cell in the 98/15 mouse hepatoma showing mitochondria (m) and ultra-mitochondria (u) of the same dimension as those in figure 3. Fixation overnight in 1 per cent osmium tetroxide in saline. Embedded in celloidin-paraffin. Magnification, X 4,800.


g
m
e
1
m
e
2
m
3
m
4

form morphology, usually in regular array, have been observed only in cells grown from tumors of known viral etiology (Claude, Porter, and Pickels, 1947; Porter and Thompson, 1948). It is significant that there is one report (Oberling, Bernhard, Febvre, and Harel, 1951) of the failure to find the ultra-mitochondria in cells of the Rous sarcoma in which "virus-like" particles may be found, and that no such "virus-like" deposits have been found in cells cultured from tumors in which no filtrable agent has been established.

Observation of cultured cells was not adequate for obtaining further information regarding the identity, internal structure and localization of the ultra-mitochondria since only the periphery of any cell is visible and the exact relation between a given cell in culture and its prototype in intact tissue is not known. Because of the obvious interest in the predominance of this particulate in cultured neoplastic cells further information has been sought from the study of similar cells in tissue sections.

The variety of satisfactory methods now available for sectioning tissue at .05–.2 μ have been recently reviewed (Selby, 1953). The features which have been found to be most important are: a vibration-free advance mechanism calibrated to advance in units at least as small as .05 μ, a motor drive to assure even operation, a liquid trough for floating the sections off the knife edge and allowing the sections to spread, and an extremely sharp knife. The advance mechanism may be achieved by adapting conventional microtomes or building new designs. Sufficiently sharp knives may be obtained by careful hand-sharpening of the conventionally-sharpened microtome knife or razor blade, or by selecting edges of plate glass which has been fractured at a 45° angle. A plastic material (n-butyl and methyl methacrylate (Newman, Borysko, and Swerdlow, 1949) which hardens by polymerization has been found to be the only embedding material which is sufficiently hard for ultra-thin sectioning. Other embedding materials such as paraffin-celloidin or ester wax which may be satisfactory for .2 μ sections undergo too much compression to be useful at greater thinnesses. Removal of the embedding from sections prior to observation introduces undesirable artifacts such as movement of unattached cell components and drying patterns as the solvent interface passes

through the cell and can be eliminated in sufficiently thin sections. For osmium tetroxide fixation of whole tissue blocks it was observed (Palade, 1952) that fixation is preceded by an acidification of the tissue and hence that it is necessary to buffer the osmic acid at pH 7.4 to assure neutral fixation. Under these optimum conditions cell and nuclear membranes appear as finely delineated membranes and the formed elements listed in table 1 are surrounded by an amorphous cytoplasmic matrix.

The internal structure of each cytoplasmic component is revealed in thin sections where only its silhouette and contour was visible in cultured cells. Mitochondria have a particularly interesting ultra-structure consisting of a regular system of internal ridges or membranes extending inwards from the limiting membrane (Palade, 1952; and Sjostrand, 1953). This characteristic internal structure makes the mitochondria readily distinguishable from other cytoplasmic components although often only a small fraction of the total mitochondrion may be present in a .05–.1 μ section. The lipid inclusions are also easily recognized because of their size, great density and usually crenated contour. Scattered through the cytoplasm lightly osmiophilic elements are distinguishable which often appear vesiculated and may vary considerably in size. Their dimension (ca. 70 mμ diameter) and presence in all cell types suggests identity with the "endoplasmic reticulum" observed in tissue culture. Another cytoplasmic component found in great profusion in pancreas, to a less degree in liver, kidney and intestinal epithelium, and even less in other cells is considered by some (Palade, 1952) to represent an array of tubules or canals and be identical with the microvesicles and endoplasmic reticulum and by others (Sjostrand, 1953) to represent cytoplasmic double membranes. The incidence of this component with cell type and its variation in quantity with feeding and starvation led Bernhard, Haguenau, Gautier and Oberling (1952) to suggest its identity with the basophilic substance or "ergastoplasm" of the cytoplasm. This view appears in accord with the facts at present available and suggests that this well-defined macromolecular structure may be the site of cytoplasmic RNA. This component appears smooth-walled in adult liver (Fig. 5), and vesiculated or beaded in embryonic liver (Figs. 6 and 7) and in the Ehrlich mouse ascites tumor (Fig. 9) where the sections are thinner.

Many of the early studies of neoplastic cells suffered from fixation artifacts and the tendency of some authors to consider any submicroscopic particle "virus-like" without recognizing the existence of normal submicroscopic cytoplasmic components. Little of morphological interest has been noted in the nucleus since chromosomes appear quite amorphous in structure following osmic acid fixation (Selby, 1953), the chromatin of the resting nucleus is also amorphous, while the nucleoli of normal and neoplastic cells show an identical "coiled" or "threadlike" morphology (Borysko and Bang, 1951; Bernhard, Haguenau, and Oberling, 1952). The nucleoli are illustrated in figures 8, 9 and 11 and the chromosomes in figures 14 and 15. In mitotic figures the only portions of the spindle which do appear as osmiophilic fibers are the chromosomal fibers (Fig. 15). The rest of the spindle appears simply as an orientation of submicroscopic particulates (Selby, 1953) and may include filaments too fine (less than 30 mμ in diameter) yet to be resolved in thin sections.

In the cytoplasm however, just as in tissue culture studies, submicroscopic distinctions between normal and neoplastic cells have been found. When the cytoplasm of cells of the mouse 98/15 hepatoma was compared to that of normal adult liver cells, it was clear that a large number of particulates, smaller than mitochondria, were present which were absent in the normal liver cells (see Figs. 4 and 8). Particle-size measurements of all cytoplasmic particles in the sectioned hepatoma were found to be identical to those made from the same tumor in tissue culture (compare Figs. 3 and 4) and indicated that the smaller

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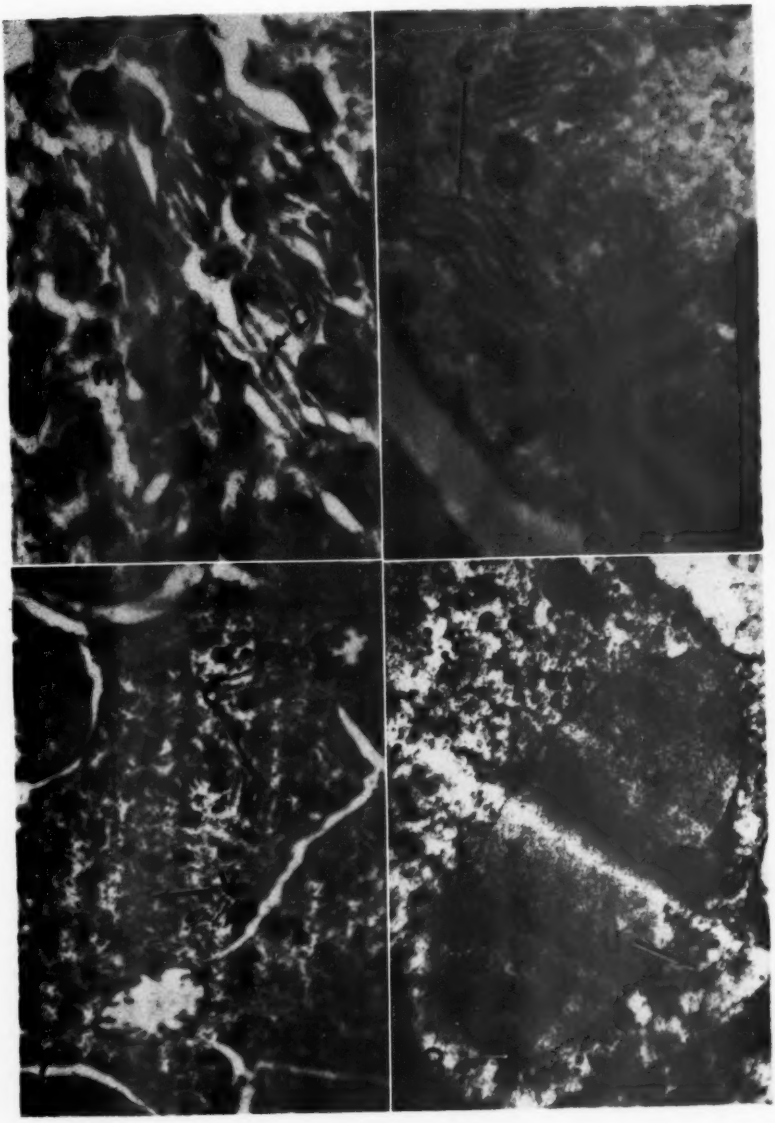
PLATE II

FIG. 5. Thin section of a portion of the cytoplasm of an adult mouse parenchymatous liver cell. Mitochondria with internal membranes (m) and cytoplasmic double membranes (c) are illustrated. The cell membrane runs across the top right-hand edge of the photograph. Fixation for 4 hours in 1 per cent osmium tetroxide buffered at pH 7.4. Methacrylate embedded. Magnification, X 78,000.

FIG. 6. Thin section of a portion of the cytoplasm of a liver cell in a full-term mouse embryo, showing the same components as in figure 5 but with the cytoplasmic double membranes (c) showing discontinuities. Fixation for 4 hours in 1 per cent osmium tetroxide buffered at pH 7.4. Methacrylate embedded. Magnification, X 11,700.

FIG. 7. Thin section of portions of several cells in the liver of a 12-week human embryo. The cytoplasmic membranes (c) are vesicular and their "doubleness" is not resolved. There is a great development of cytoplasmic microvesicles (v). Two canaliculi with surface microvilli are illustrated. Fixation for 4 hours in 1 per cent osmium textroxide buffered at pH 7.4. Methacrylate embedded. Magnification, X 7,800.

FIG. 8. Thin section of a cell of the 98/15 mouse hepatoma. "Coiled" nucleoli, mitochondria, ultra-mitochondria (u) and a lipid inclusion are illustrated. Fixation overnight in 1 per cent osmium tetroxide in saline. Celloidin-paraffin embedded. Magnification, X 4,800.



particles had an average diameter of 120 mμ independent of that of the mitochondria. Although the fixation of the hepatoma cells was unfortunately poor (because of the large amount of free lipid present in these tumors) so that the internal structure of the smaller particulates could not be examined, they did appear to be the same ultra-mitochondria as observed in tissue culture rather than simply small mitochondria. Cells of embryonic liver (Figs. 6 and 7), were found to contain the same cytoplasmic components as normal liver, showing only a greater development of the microvesicles. Osmiophilic particles were, of course, observed in the 120 mμ size range but these appeared on the basis of size distribution to be small mitochondria or small sections of mitochondria and not the characteristic ultra-mitochondria. The ultra-mitochondria have also been observed in sections of the same Jensen rat sarcoma in which it was first observed in tissue culture (Porter and Kallman, 1952). The distinction between cultured neoplastic and normal cytoplasm has been upheld for cells of these two tumors at least *in situ* and our distinction between embryonic and neoplastic cytoplasm has been upheld in the case of liver and hepatoma.

Because of the poor fixation generally obtained of tumors in whole tissue, attention was turned to the ascitic form of two mouse tumors: the Ehrlich mouse ascites tumor and an ascites form of the sarcoma 180 tumor. These tumors proliferate in the peritoneal cavity of mice producing an ascitic fluid containing a very high percentage of malignant cells. Immediately upon removal from the animal the cells are fixed in osmic acid in balanced salt solution for 30–60 minutes. After the osmic acid is washed free by repeated centrifugation, fixation in formalin may follow to harden the tissue (this step is op-

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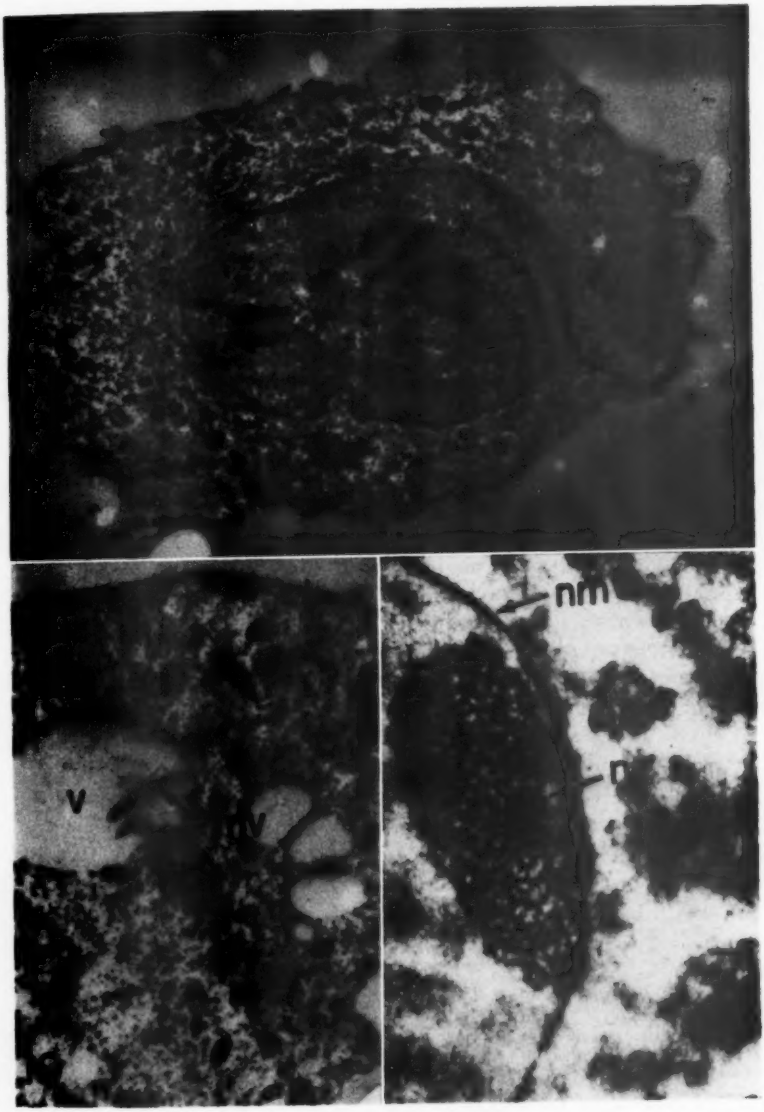
PLATE III

All cells of the Ehrlich mouse ascites tumor were fixed from 30–60 minutes in 1 per cent osmium tetroxide at pH 7.2 and embedded in methacrylate. The embedding was not removed from these or any sections illustrated here.

FIG. 9. Thin section of a typical cell of the Ehrlich ascites tumor showing nucleoli, and normal cytoplasmic components. Magnification, X 4,800.

FIG. 10. Thin section of a portion of a vacuolated Ehrlish mouse ascites tumor cell showing "microvilli" (mv) from the surface of a vacuole (v) similar to those commonly found at cell surfaces, as in the top of this photograph. Mitochondria and cytoplasmic microvesicles are illustrated. Magnification, X 10,000.

FIG. 11. Thin section of the nucleo-cytoplasmic boundary of a cell in the 98/15 mouse hepatoma to illustrate the "coiled" nucleolus (n) and double nuclear membrane (nm). Fixation overnight in 1 per cent osmium tetroxide in saline. Embedded in celloidin-paraffin. Magnification, X 12,000.


nm
v
v

tional). The cells are then dehydrated and embedded in a pellet by repeated centrifugation and suspension. Optimum penetration of fixative and embedding is thereby achieved in these essentially single cell preparations.

As illustrated in figures 9 and 10, the cytoplasm of the Ehrlich ascites tumor contains the usual cytoplasmic components, nucleoli are "coiled," nuclear membranes commonly appear double, and quite uniform-diameter "microvilli" project from the free cell surface and from the surface of vacuoles inside the cell. Occasional small osmiophilic particles in the 120 mμ size range appear to be, as in the case of embryonic liver, simply small segments of mitochondria although analysis of their structure at higher resolving powers has not yet been made. Thus the cytoplasm of the Ehrlich ascites tumor cells did not appear significantly different from that of normal cells studied until a very unusual cytoplasmic inclusion was noted. In a few cells found at random in a given specimen or among a variety of specimens, clusters of constant-diameter spheres in very regular array were noted. These are illustrated in figures 12 and 13. The individual particles are about 40 mμ in diameter and generally aggregate in exact hexagonal array. No enclosing membrane is evident but isolated groups throughout the cytoplasm appear, by their alignment, to be organized into a larger single unit in the whole cell. About a year following this observation, another investigator in our laboratory[1] noted the same particles in specimens obtained from a different animal laboratory of our Institute so that the possibility of a chance viral contaminant has been reduced. These particles have now also been seen in cells of all stages of

[1] Clifford E. Grey and John J. Biesele—unpublished observations.

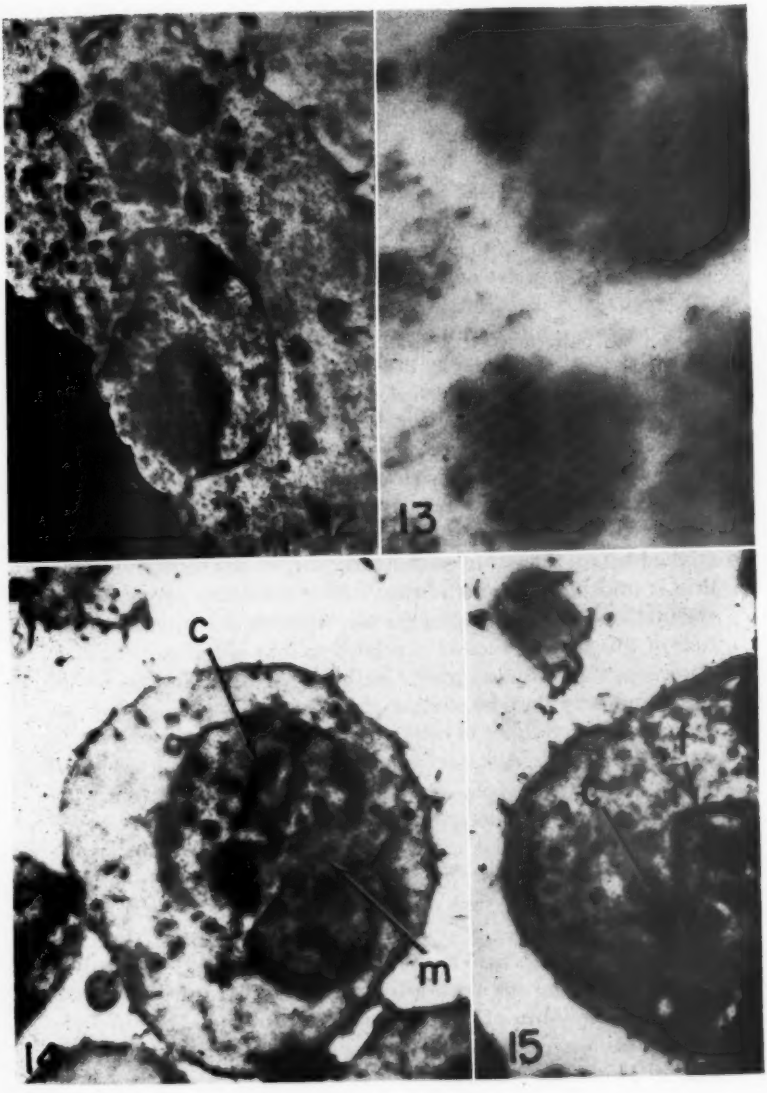
PLATE IV

FIG. 12. Thin section of a portion of cell of the Ehrlich mouse ascites tumor. Pseudo-crystalline arrays of constant-diameter spheres (s) are visible in the top left-hand corner. Vertical streaks are artefacts due to dirt on the photographic plate. Magnification, X 7,800.

FIG. 13. Enlarged portion of figure 12 showing the clusters of 40 mμ particles in hexagonal array. Magnification, X 42,000.

FIG. 14. Thin section of a cell of the Ehrlich mouse ascites tumor in late prophase. The nuclear membrane is partially dissolved, the chromosomes (c) have taken form and mitochondria (m) are visible migrated into the nuclear region in close proximity to the chromosomes. No internal structure may be seen in the chromosomes even at much higher magnifications. Magnification, X 4,200.

FIG. 15. Thin section of another mitotic cell from the same specimen as that of figure 14. The cell is in anaphase with the chromosomal fibers (f) and separating chromosomes (c) visible. Magnification, X 4,200.


13
c
m
14
15

development from mitotic to degenerating cells. They have not yet been observed in cells of the sarcoma 180 ascites tumor. Studies of the solid form of the Ehrlich ascites tumor are at present underway.

The observation of truly "virus-like" deposits in a tumor for which no viral etiology has yet been proven need only suggest the possibility that this particular tumor may have a growth mechanism different from that of all the other tumors examined in which such inclusions are not found. Since the ultra-mitochondria has not yet been clearly identified in the Ehrlich ascites cells, the possibility exists that, just as in the case of the Rous sarcoma, these uniform-diameter particles do represent some agent involved in the growth of this tumor in whose presence the ultra-mitochondria are absent. Such an agent is currently being sought in biological experiments in our laboratory.[2]

Despite the fact that so few tumors have yet been adequately studied in thin sections, it is clear that much interesting information has already been revealed concerning cytoplasmic structures specific to tumor cells. Many more tumors must, of course, be studied before the nature and significance of the ultra-mitochondria is understood and studies at higher resolving power will be essential to determine, once for all, whether the internal structure of this smaller moiety is related to that of the more normal mitochondria. Its development with growth and proliferation of the tumor may then be studied and correlated with parallel biochemical data. Studies of cells in other conditions of hyperplasia should then be undertaken to determine a more exact relation of these submicroscopic characteristics to neoplasia. With the development of thin-sectioning techniques for routine use, such submicroscopic cellular criteria as these which are beyond the scope of optical microscopy and pathology may then be applied to practical problems such as elusive diagnoses and evaluation of therapy.

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[2] Charlotte Friend and Cecily C. Selby—unpublished observations.

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THE DOSIMETRY OF BETA RADIATION AND ITS APPLICATION TO INHOMOGENEOUS DISTRIBUTIONS OF RADIOACTIVITY IN CELLS

W. K. SINCLAIR*

The use of radioactive isotopes in medicine and biology on a wide scale has caused a great deal of attention to be focussed on the dosimetry of beta- and gamma-radiations; that is the calculation of dose in ergs per gram or in roentgens when the radioactive material is distributed in various different ways. When radioisotopes are used therapeutically, these calculations will be of major importance as they control the amount of material that should be given to a patient. In diagnostic uses or in tracer applications the dosimetry may assume a relatively minor role in that it is used only to decide that the dosage level employed is not a dangerous one or is unlikely to upset the course of the investigation by damaging the tissues involved.

In most of these circumstances it is usual to calculate an average dose for the particular region of tissue under study. The distribution of dose throughout the region will, however, rarely be uniform, and the variations in dose may be as important, or more important than the average dose. This is frequently the case with radium implants and in many circumstances when beta emitting material is distributed in tissue. The heterogeneity in these two cases is often not dissimilar (Mayneord and Sinclair, 1953). In the beta case, however, the short range of the particles may even lead to significant differences in dose distribution between different cells or groups of cells. In this report, the main features of gamma and beta dosimetry are outlined and some results of the latter are applied to the estimation of variations in dosage rate resulting from inhomogeneous distributions of activity in cells.

Gamma Dosimetry

In the dosimetry of gamma-radiation, the dimensions of the region or body under study are always small compared with the

* On leave from the Physics Department, Royal Cancer Hospital, London, England. Presented at the Seventh Annual Cancer Symposium, May, 1953, at the M. D. Anderson Hospital for Cancer Research, Houston.

gamma-ray range. The absorption of gamma-radiation is small and may either be neglected or approximate methods may be used to estimate its value. Thus, if the dosage rate at unit distance from a point source of 1 mc of gamma-emitter (often denoted by k) is known, the problems of determining the dose due to various distributions of material are largely mathematical.

The value of k for quanta of energy E can be estimated from a knowledge of the real energy absorption coefficients for that energy.

k = Dosage rate in r/hour at 1 cm from a point source of 1 mc.

$$= \frac{\text{Intensity at 1 cm}}{\text{Energy flux per roentgen}}$$

$$= \frac{\frac{1}{4\pi} \times 3.7 \times 10^{7} \times 3600 \times 1.6 \times 10^{-6} \times E}{Ef}$$

where E is the energy of the quantum in Mev
Ef is the energy flux per roentgen

$$= \frac{\text{Energy absorbed per gram}}{\text{Absorption coefficients per gram}}$$

$$= \frac{83.7}{(\sigma_{\alpha} + \pi_{\alpha} + \tau)} \text{ ergs}$$

$$\therefore \; k = 201.2 \; (\sigma_{\alpha} + \pi_{\alpha} + \tau) \, E$$

The value of k for each quantum can then be used to calculate its value for each isotope provided the disintegration scheme of the isotope is known. The study of disintegration schemes by spectrometry and the advance of counting and ionization methods of standardizing radioactive sources has enabled values of k for most of the medically important isotopes to be established. In general, they range between 1 and 20 r/hour per mc at 1 cm. (Mayneord and Sinclair, 1953)

The calculation of the dosage rate in and about gamma emitters of simple geometrical shape, such as the line, ring, disc, cylinder, ellipsoid and sphere are well known (Evans, 1948; Mayneord, 1950; Parker, 1938; Sievert, 1921; Sievert, 1932; and Wilson, 1945). Many of these have been confirmed experimentally. For example, in the case of a sphere of radius 'a' uniformly filled with radioactive material of specific activity ρ mc/cc the maximum dosage rate, at the centre, is

$$4 \pi \kappa \rho a$$

while at a distance 'c' from the centre it is

$$\pi \kappa \rho \left(2a + \frac{a^2 - c^2}{c} \log \frac{a + c}{a - c} \right)$$

(Souttar, 1931)

The distribution of dosage rate with distance is shown in Figure I. If the distribution of material in the sphere is not uniform, the variations produced in dosage rate are usually quite small.

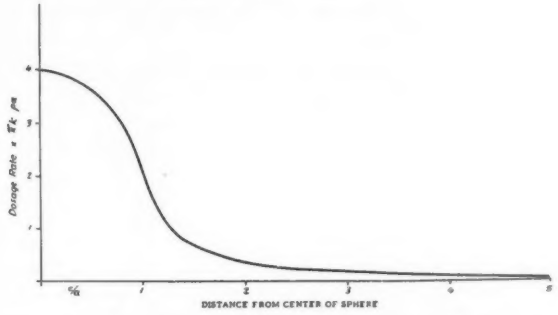


FIG. I. Distribution of Dosage Rate from a Uniformly Filled Radioactive Sphere of Gamma Emitter.

Beta Dosimetry

The short range of the beta radiation simplifies the problem of estimating the dose for a homogeneous distribution of material because the energy absorbed in the region is now equal to the energy emitted provided the dimensions of the region exceed the beta range. The calculation may then be made as follows:—(Marinelli, 1942)

Energy absorbed per gram = Energy emitted per gram
$= 3.7 \times 10^7 \times \rho \times \bar{E}$ Mev/sec/gm
where $\bar{E}$ is the average energy of the beta radiation
and ρ is the specific activity in mc/gm
$= 3.7 \times 10^7 \times 3600 \times 1.6 \times 10^{-6} \rho \bar{E}$ ergs/hour/gm

$$= \frac{3.7 \times 10^7 \times 3600 \times 1.6 \times 10^{-6} \rho \bar{E}}{93} \text{ r/hour in soft tissue}$$

$= 2280 \rho \bar{E}$ roentgens/hour

The total dose delivered if this activity stays in the tissues indefinitely is obtained by multiplying this by the average life of the atoms.

$$\text{Total Dose} = 2280 \rho \bar{E} \times \frac{T}{0.693} \times 24 \text{ roentgens for T in days}$$

$$= 79{,}500 \rho T \bar{E} \text{ roentgens}$$

This simple expression of the dose is frequently inadequate, particularly as the distribution of activity is rarely homogeneous and the range of the beta particles may not be large enough to sustain uniformity of dosage as is often possible with gamma emitters. At the clinical level the effect of this inadequacy in dosage estimation is frequently evident. It seems to require much higher doses of I–131 in the thyroid, or of Au198 injected interstitially to produce the same effect as would have been produced by relatively low doses of external radiation. On the other hand, in producing whole body effects, P–32 administered intravenously appears to be somewhat more effective than X-radiation for the same average dose. Clearly these differences are not due to any imagined difference between "beta" roentgens and "gamma" roentgens, since each represents the same amount of energy absorption, but they indicate an inadequacy in our expression of the average dose throughout a region.

In attempting to analyze the situation further we have been handicapped by lack of information regarding the distribution of ionization about a point source of a beta emitter. A good deal of attention is now being paid to this problem, but theories of multiple scattering are complex and unsatisfactory, and experimental studies of the problem at very short distances are difficult. Loevinger (1950) has recently employed an indirect experimental approach by using an extrapolation ionization chamber to measure the distribution of ionization from the surface of a thin infinite plane of beta emitter, conditions which can relatively easily be satisfied in practice. Empirical relations were fitted to the experimental curve and these relations differentiated

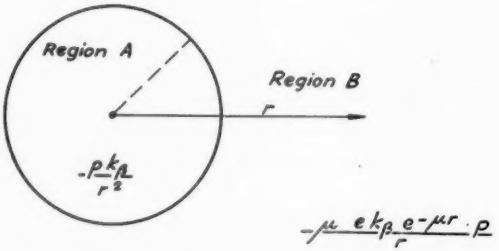


FIG. II. Distribution of Dosage Rate About a Point Source Beta Emitter. (Activity p mc)

to give the distribution about a point source. Loevinger found that for P–32 the point source function could be represented by the inverse square law alone, up to a distance of $^1/\mu$ and beyond this distance a diffusion law $e^{-\mu r}/r$ applied. The coefficient μ is, in this case, the slope of the exponential region of the experimentally determined plane source distribution. It is not a coefficient of real energy absorption (Fig. II).

If a new constant $\kappa\beta$ is defined as the dosage rate in roentgens/ hour at unit distance from a point source of 1 mc of beta emitter (following the analogy with gamma radiation), the dosage rates around the point source can be represented as in figure II, where the value of the constant in region B is obtained from the necessary conditions for continuity at the joining point. Clearly, if these relations hold, the total dosage rate emitted from a source of ρ, mc is equal to that absorbed in region A and in region B.

$$\text{Total energy absorbed in region A} = \int_0^{\frac{1}{\mu}} \rho . 4\pi r^2 \left(\frac{\kappa\beta}{r^2}\right) dr$$

$$= \frac{4\pi \kappa\beta \rho}{\mu}$$

$$\text{Total energy absorbed in region B} = \int_{\frac{1}{\mu}}^{\infty} \rho . 4\pi r^2 \left(\frac{\mu e \kappa\beta e^{-\mu r}}{r}\right) dr$$

$$= \frac{8\pi \kappa\beta \rho}{\mu}$$

$$\text{Total energy absorbed from } \rho \text{ mc} = \frac{12\pi \kappa\beta \rho}{\mu}$$

and this, as we have seen already, must equal $2280\,\rho\bar{\epsilon}$. The value of $\kappa\beta$ is thus established when μ is known. For P–32 for example, with $\mu = 9.1$ cm^2/gm, $\kappa\beta$ is of the order of 385 r/hour per mc at unit distance.

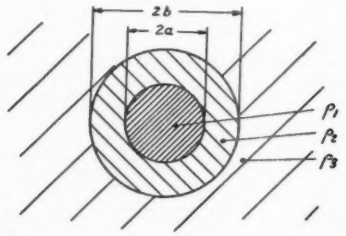
Alternatively the value of $\kappa\beta$ could be established from fundamental principles following the same method as for k for gamma-radiation. In this case, $\kappa\beta = 181\,\mu a^{\bar{\epsilon}}$ roentgens/hour in soft tissue and μa^{ϵ} is the same quantity as dE/dx used by Heitler.

(1936). Values calculated over a range of energies show that $\kappa\beta$ varies by only 20 per cent between 0.5 and 10 Mev and has an average value of about 350 roentgens/ hour/mc. (Mayneord and Sinclair, 1953).

It should be emphasized that the relations given above were established by empirically fitting functions to experimental data and apply better to high energy beta emitters than to low energy beta emitters. Loevinger has since extended his theory to account more exactly for the experimental data over a range of beta energies but the modifications have added considerable complexity to the form of the functions. For the purposes of this discussion it will be assumed that the simpler relations hold.

Application of Beta Dosimetry at Cellular Levels

Single Cell. Suppose we consider first a single cell, spherical in shape, of radius b, containing a spherical nucleus of radius a, surrounded by tissue fluid. Let us assume a concentration $\rho 1$ mc/cc in the nucleus, $\rho 2$ in the cytoplasm and $\rho 3$ in the surrounding fluid. The dosage rate will clearly vary as we progress from the center of the nucleus outwards across the cell boundary until a distance equal to the beta range from the cell is reached. The dosage rate at any point will be made up of three contributions from each of these separate concentrations. If we assume $\rho 1 > \rho 2 - > \rho 3$, then the dose will be a maximum at the center of the nu-



The dosage rate at a point P distant c from the center of the cell.

= Dosage rate due to uniform concentration ρ_3
+ Dosage rate due to extra concentration $\rho_2 - \rho_3$ in cytoplasm.
+ Dosage rate due to extra concentration $\rho_1 - \rho_2 - \rho_3$ in the nucleus

FIG. III. Calculation of Dose in a Single Cell.

cleus and the dose at any point c from the nucleus will be

$$\frac{12\,\pi\,\kappa\beta\,p_2}{\mu} + \pi\,\kappa\beta\,(p_2-p_3)\left\{2\,b + \frac{b^2-c^2}{c}\,log\,\frac{b+c}{b-c}\right\}$$

$$+ \pi\,\kappa\beta\,(p_1-p_2-p_3)\left\{2\,a + \frac{a^2-c^2}{c}\,log\,\frac{a+c}{b-c}\right\}$$

To fix the relative magnitudes of these three contributions, it is necessary to take particular values. Suppose $\mu = 20$ cm²/gm for nuclear beta particles of maximum energy 1 Mev (for example Au–198), and that

$$a = 2.5 \text{ microns}$$
$$b = 5.0 \text{ microns}$$

and that $\rho_1 = 10\ \rho_2 = 1{,}000\ \rho_3$.

The relative contributions to the total dosage rate will then be, in units of $\pi\,\kappa\beta\,\rho_3$ as plotted in figure IV.

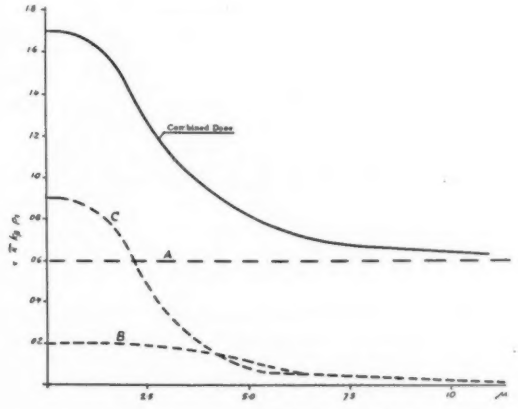


FIG. IV. Dose in an Individual Cell.

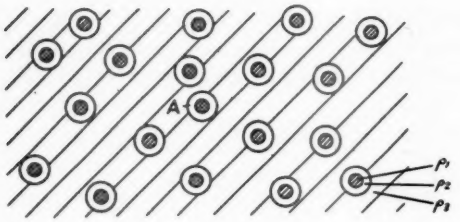
Cell Surrounded by Others. If the individual cell considered is surrounded by other cells of similar nature and concentration, the contribution to the dosage rate due to the other cells can be estimated in the same way and will evidently be the same to all points of the cell.

The additional dosage rate due to the concentration in the cytoplasms

$$= \frac{12 \pi \kappa\beta \, (p_2 - p_3)}{\mu} \; x \; n \; v$$

Additional dosage rate due to the concentration in the nuclei

$$= \frac{12 \pi \kappa\beta \, (p_1 - p_2 - p_3)}{\mu} \; x \; n \; v_n$$



Suppose n cells/unit volume

v volume of each cell

v_n volume of each nucleus

Dose due to the other cells

= Dose at A due to the concentration in the cytoplasms of the other cells

+ Dose at A due to the concentration in the nuclei of the other cells

FIG. V. Calculation of Dose in a Single Cell Surrounded by Other Similar Cells.

n = number of cells per unit volume
v = volume of each cell
v_n = volume of nucleus

The dosage rate will now depend on the volume $n v$ occupied by the cells. If we take $n v = 0.8$ as an average figure for the body, $n v_n$ will be about 1/8 of this, so that with $p_1 = 10 \; p_2 = 1{,}000 \; p_3$ as before, the additional dosage rates due to cytoplasm and nuclear concentration are about equal and each has the value about $50 \; \pi \kappa\beta \; p_3$. Thus, under these conditions the dosage rate received by a cell is almost entirely due to those round about it, and only about 1 per cent is due to its own nuclear and cytoplasmic concentrations. In other words, the distribution of dosage rate is substantially uniform, as might have been expected for the conditions assumed.

Suppose, however, that instead of all the cells about A containing the same concentrations as A, only 10 per cent of them do so, (*i.e.* $n v = 0.08$) while the remainder contain only p_3. The single cell A will then contribute 10 per cent of its own dosage rate. If the number of cells about A contain-

ing the same activity were only 1 per cent of the total number (*i.e.* $nv = 0.008$), then the maximum dosage rate at A would be equally shared between the contribution from its own concentrations and from the cells about it.

The dosage rate due to the extra concentrations in the cell is not dependent on μ as long as $\frac{1}{\mu}$ exceeds the cell radius. The dosage rate due to the neighboring cells decreases as μ increases. μ Increases as the beta energy decreases and for C-14 (E max = 0.145 Mev) is about 350 cm²/gm. For H-3 (E max = 0.018 Mev) μ may be 10 times larger than this. The dosage rate due to its neighbors is given in table 1 for a number of values of μ &

TABLE 1

RELATIVE DOSAGE RATES IN A SINGLE CELL SURROUNDED BY OTHERS OF SIMILAR CONCENTRATION

Suppose $\rho_1 = 10\rho_2 = 1000\rho_3$

Dosage rates in units of $\bar{n} k_\beta \rho_3$

Volume occupied by cells	Dosage rate due to cell itself	Dosage rate due to other cells		
		$\mu = 20$	$\mu = 200$	$\mu = 2000$
$nv = 0.8$	1.1	100	10	1.0
$nv = 0.08$	1.1	10	1.0	0.1
$nv = 0.008$	1.1	1.0	0.1	0.01

nv. These show that significant variations in dosage rate are likely to result from different distributions, particularly for low energy beta emitters, even at the cellular level.

Applications

The calculation of dose for a single cell is of rather limited application. It applies to circumstances where a cell contains radioactivity and is separated from other cells containing activity by a distance greater than the beta range. This may happen to isolated cells in the thyroid or to floating macrophages containing Au–198 in the peritoneal or pleural fluid.

The calculations for a cell surrounded by others may be used to estimate variations in dosage rate due to I–131 in the thyroid, Au–198 used interstitially, or P–32 administered intravenously. It is quite well known autoradiographically that concentration differences of 10:1 and sometimes 100:1 are possible in the case

of I–131 and Au–198. It should be possible to combine this analysis with densitometer measurements of autoradiographs in order to assess the variations in dosage rate over regions of tissue. This type of calculation may also be very useful in assessing the dose due to labelled tracers, particularly for C–14, S–35, or H–3, where the label is attached to a specifically localizing compound.

The examples chosen were those in which the nucleus has the largest concentration. It is evident that the same method of analysis could, however, be used for cases where the cytoplasm or the extra-cellular fluid contained the greatest concentration of activity. It is clear that some means such as this for assessing the dosage distribution at the cellular level is very necessary before the radiosensitivity to beta radiation of different cells or parts of a cell can be compared.

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NUCLEOPROTEINS IN THE MITOTIC CYCLE

HEWSON SWIFT*

One current trend in the study of cells has been an increasing interest in the chemical composition of various cell structures, such as nucleoli and chromosomes. From studies of cell chemistry it has become clear that the picture of a fixed and stained cell that we know as histologists or pathologists, is largely a nucleic acid and protein picture. The eosin, for example, in greater part stains protein, and the hematoxylin, the nucleic acids. Chromatin, and the chromosomes are, of course, so called because of their affinity for basic dyes, and this affinity is a function of their nucleic acid content. Many cell chemists have been interested in nucleoproteins, then, simply because these are major constituents of cells. If we seek an answer to such questions as: "What is the chemical nature of the gene?", or "What changes take place in cells during normal or abnormal growth?", studies of the nucleoproteins seem likely to give us a certain amount of insight. If our observations, furthermore, are quantitative, as I hope you can see from this discussion, we can often draw more conclusions from the data we obtain.

Methods. Nucleoproteins have been studied in a good many different ways. The data presented here have been obtained with one tchnique, which has recently been called cytophotometry (Moses, 1952)—the study of cells through their capacity to absorb light of various wavelengths. This technique, as modified from Pollister and Ris (1947), is shown in simplified form in figure I. The specimen is placed on the microscope stage. Light of one particular wavelength is isolated by the monochromator and this is passed through the microscope. The cell to be studied is selected, and centered in the field. The cell image, magnified several hundred times, is projected on a diaphragm, arranged so that light only from a small piece of the cell, from 0.5 to 6 microns across, falls on the sensitive phototube. Thus the light absorbed, for example, by a single nucleolus or part of one chromosome, may be measured.

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By changing the wavelength of light it is, of course, possible to obtain absorption curves for particular cell regions. For much of the ultraviolet region quartz or reflecting optics must be used. The technique is very simple in principle, but in practice often involves rather expensive equipment and a certain knowledge of the physical systems involved. There are also a great many variables, some chemical, some optical, some electronic, that may make the data obtained inaccurate and misleading (Swift, 1953 for review). Photometric instruments then, must be carefully calibrated, and the material to be studied, adequately prepared.

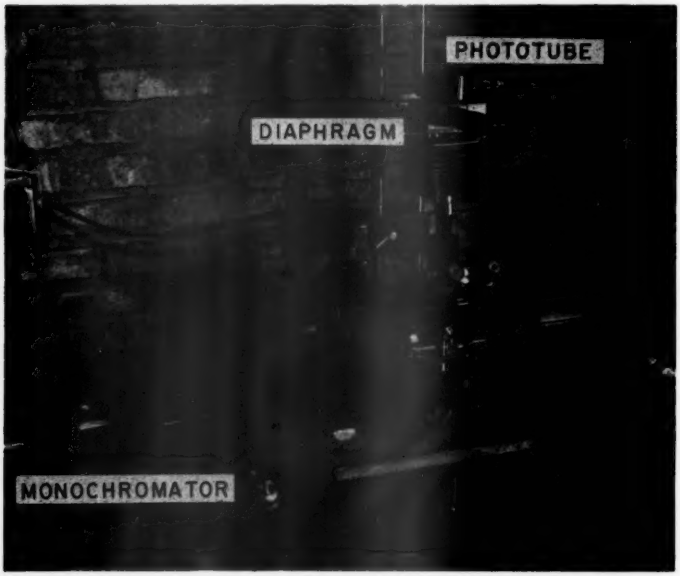

FIG. I. Photograph of a simple measuring instrument, showing major components.

Suppose, for a moment, that we take such an instrument and run absorption curves with ultraviolet light on various cell regions. What can these measurements tell us of their nucleoprotein composition? Figure II shows data from two different salamander cells, an oocyte at the left, and a red blood cell at the right. Curves taken through the regions of chromatin both show peaks at 260 mμ, characteristic of nucleic acid absorption. A similar peak is shown by the nucleolus and cytoplasm of the oocyte. If we measure the nuclear sap of the oocyte, away from the chromosomes, we obtain a peak between 270 and 280 mμ, characteristic of the tyrosine and tryptophane in the sap proteins. The nucleic acid peak is absent. There is

also no nucleic acid peak in the red blood cell cytoplasm, indicating, as we might expect, that no measurable nucleic acid occurs. One can also see that there is a protein side-peak in the oocyte chromatin, but one is not noticeable in the red blood cell. This means that the nucleic acid to protein ratio is very different in these two measured regions.

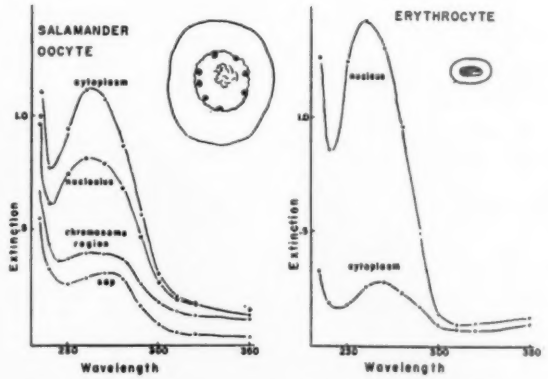

FIG. II. Ultraviolet absorption curves of salamander cells. Left, from acetic-alcohol fixed *Triturus* oocytes. Right, from an unfixed *Ambystoma* erythrocyte. (Data from Rasch and Swift, unpublished.)

The nucleic acids are, of course, of two types, named for the different sugars they contain—desoxyribose nucleic acid, or DNA, and ribose nucleic acid, or RNA. Since these are quite different compounds chemically, it is possible to extract one, by acid or enzyme treatment, and to leave the other in place. This fractionation can be visualized in figure III. Here lily ovary cells have been stained with the basic dye Azure B—a component of the Giemsa stain. As shown by Flax and Himes (1952) the DNA with Azure B stains blue-green, and the RNA, purple. After ribonuclease we have only the blue-green DNA remaining, and this only in the chromosomes, and the interphase chromatin. After desoxyribonuclease only the purple component remains, localized in the nucleoli and cytoplasm. Notice that the nuclei themselves do not stain at all, even though they contain considerable amounts of RNA. This is a good example of the fact that, in some cases, basic stains may not give an accurate picture of nucleic acid localization.

The influence of these enzymatic extractions on absorption curves of chromatin from a lily nucleus can be seen in figure IV. Desoxyribonuclease removes a large portion of the chromosomal nucleic acid, but some still remains. This is removable by ribonuclease, leaving, finally, only a typical protein absorption curve. By computing the amount of absorbing substance

extracted by the enzyme, we can determine, under suitable conditions, the DNA, RNA, and protein present in the structure studied.

In addition to ultraviolet absorption, certain chemical reactions that give visible colors with cell constituents can also be used. Two of these that have proved useful are the Feulgen reaction for desoxyribose, which thus gives us the location of DNA, and the Millon reaction for tyrosine. Both reactions are subject to variables which affect their intensity (Swift, 1953; Rasch and Swift, 1953). In the case of the Feulgen reaction these variables may be controlled in many cases, so that amounts of DNA in nuclei may be easily and accurately determined.

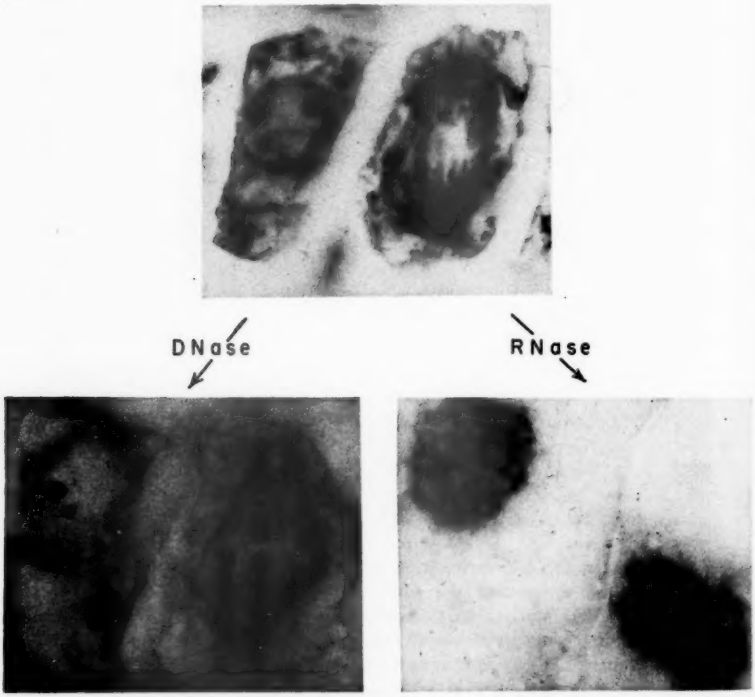

FIG. III. Lily ovary wall cells fixed in acetic-alcohol and stained with Azure B at pH 4.0. In untreated cells dye is bound by chromosomes, nucleoli, spindles, and cytoplasm. After desoxyribonuclease interphase nuclei are unstained except for nucleoli, and spaces in spindle occupied by chromosomes are clear. After ribonuclease only chromosomes and interphase chromatin are stained; nucleoli, spindle, and cytoplasm are unstained.

Results

These, very briefly, are the techniques we have been using. If we put them together we get the nucleic acid and protein picture illustrated in figure V. Chromosomes and interphase chromatin contain DNA, RNA, and proteins. There are at least two major chromosomal protein fractions, but these we have not distinguished in the present studies. Nucleoli contain RNA and protein, and the nuclear sap, at least in some cases, only protein as also found by Caspersson (1950) and Brown, *et al.* (1950). The cytoplasm in these lily cells contains RNA and protein; in some special cases the RNA may, of course, be lacking.

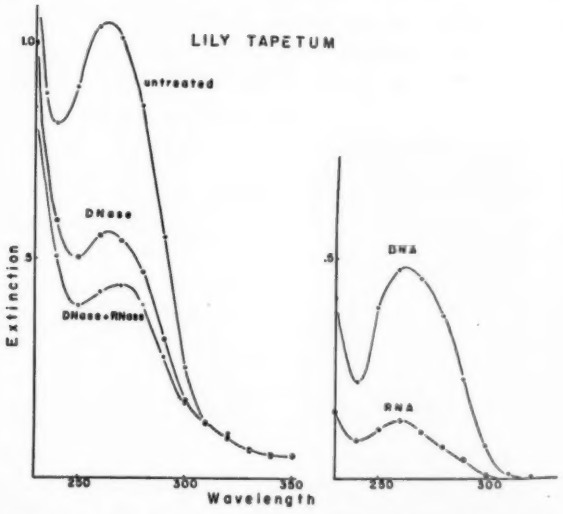

FIG. IV. The effect of desoxyribonuclease and ribonuclease on ultraviolet absorption curves of a tapetal nucleus of *Lilium* henyri. Curves at right are obtained from those at the left by subtraction, and indicate absorption of the material removed. Material extracted by desoxyribonuclease shows a curve slightly broader than that of pure DNA, so presumably the enzyme preparation also removed a small amount of protein. (Data from Rasch and Swift, unpublished.)

DNA. It is pertinent to discuss the ways that some of these different fractions vary from tissue to tissue. We have been

particularly interested in the DNA, mostly as determined in Feulgen-stained nuclei. If we take, for example, a slide of Feulgen-stained mouse liver tissue, and measure the nuclei at random, taking care only to measure whole nuclei, uncut in the sectioning procedure, the distribution of values is strikingly non-random (Fig. VI). All measurements fall into three clearly defined classes, with the means in a 1:2:4 ratio. Similar classes occur in the pancreas, although the kidney tubule nuclei and spinal cord motor neurons all fall into the lowest class. Since mammalian liver is known to contain polyploid cells, with 4 and 8 times the normal chromosome number (Biesele, 1944), it seems probable that the DNA amounts parallel the polyploid condition. The haploid spermatids, with half the diploid chromosome number, average almost exactly half the lowest class. Figure VII shows similar values for corn plant leaf and pollen nuclei. There are two classes, apparently diploid and tetraploid,

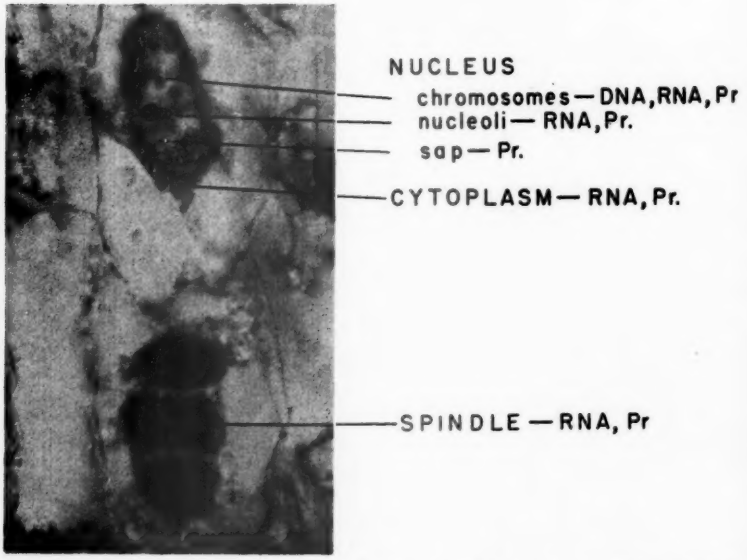

FIG. V. Ovary wall nuclei from *Lilium henyri* stained as in figure III. Prophase at top, telophase at bottom. Note the strongly basophilic telophase spindle, associated with the high concentration of ribose nucleoproteins.

in the leaf. The haploid sperm nuclei average half the diploid class.

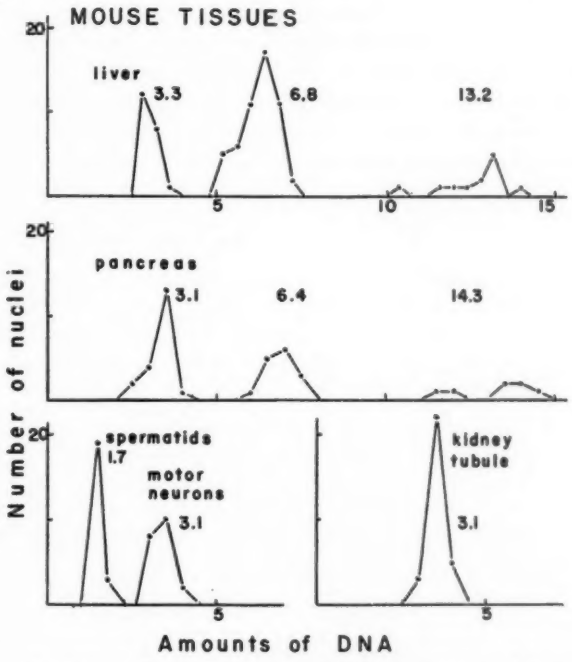

FIG. VI. Amounts of DNA in individual mouse nuclei, as determined with the Feulgen reaction. Each unit is equal to approximately 2×10^{-9} mg. DNA. (Data redrawn from Swift, 1950a, with a few added measurements.)

We have found these relationships to hold for several thousand measurements on a variety of plant and animal species. These results can be summarized by saying that, in any one individual animal or plant, there appears to be a constant relationship between the number of chromosomes present, and the DNA. Even as there is a constancy in *chromosome numbers* in all diploid cells of an organism, there is also a constancy in the *amounts of DNA per nucleus*. This, of course, also fits the geneticist's concept that a complete genome exists within each

nucleus, so that we can suggest, as did Boivin in 1948, that the DNA is an essential component of the gene—that its quantitative stability, in these tissues, is possibly a reflection of the stable, non-blending, character of the genotype. The small normal variation in chromosome number in some tissues, recently described by Hsu (1952) would be expected to cause small variations in the amounts of DNA in nuclei, but certainly not to contradict the general concept of a constant relationship between chromosomes and DNA.

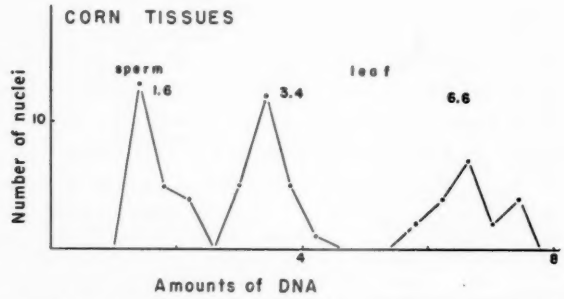

FIG. VII. Amounts of DNA in individual corn nuclei, as determined with the Feulgen reaction. Sperm nuclei were measured from sections of mature pollen. (Data redrawn from Swift, 1950b.)

The values shown so far all have been from tissues where cell divisions are rare. What happens to the DNA when a nucleus divides? Figure VIII shows the course of DNA amounts during the mitotic and intermitotic cycle of an onion root. The division figures here had to be measured by a special technique involving two different wavelengths, so that variation caused by the irregular distribution of the absorbing material could be cancelled out (Ornstein, 1952; Patau, 1952). Here two points are of interest: First, the DNA is synthesized in nuclei falling more or less into one volume class, some time about the middle of the intermitotic growth period; and, second, during the entire course of mitosis itself there is no significant change in amounts of DNA—other than the obvious partitioning to the two daughter nuclei.

We have obtained similar data from other dividing plant and animal tissues (Swift, 1950a and b; Alfert and Swift, 1953). In these cases DNA synthesis, involving very nearly an exact doubling, occurs some time in interphase, before prophase begins. The one exception we have found is in pollen formation in the plant *Tradescantia*, where synthesis takes place in the early meiotic prophase (Swift, 1950b).

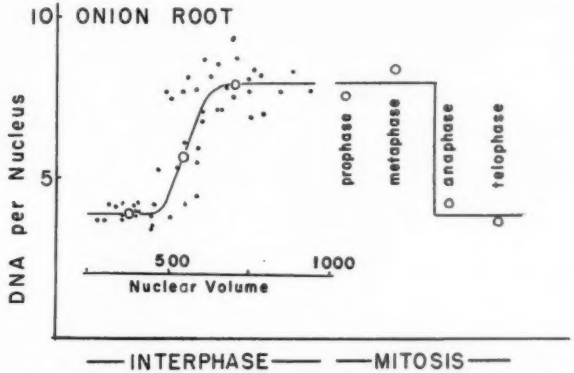

FIG. VIII. Amounts of DNA (Feulgen) in individual nuclei of onion root meristem plotted against nuclear volume in cubic microns at left (as suggested by Patau). The abscissa at right was determined merely by cytological observation of the mitotic stage, and bears no close relation to the time sequence involved. Large circles represent means of three or more values. Means for the mitotic stages were determined with the two wavelength method, and are from Patau and Swift (1953).

In a few tissues the chromosomes are condensed and visible during interphase, and so we can see what effect this doubling process has on chromosome structure. In figure IX oogonial nuclei are shown from a grasshopper ovary before and after DNA synthesis—the chromosomes in both cases appear double, but after synthesis have increased in thickness.

As another indication that DNA synthesis takes place in interphase, preceding cell division, we can see how DNA values change during the early stages of regeneration in rat liver. Figure X shows the distribution of values in normal rat liver, as compared with a liver 18 hours after about 70 per cent of the

tissue had been removed. The mitotic index of the remaining liver had not yet started to increase, although DNA synthesis had obviously begun.

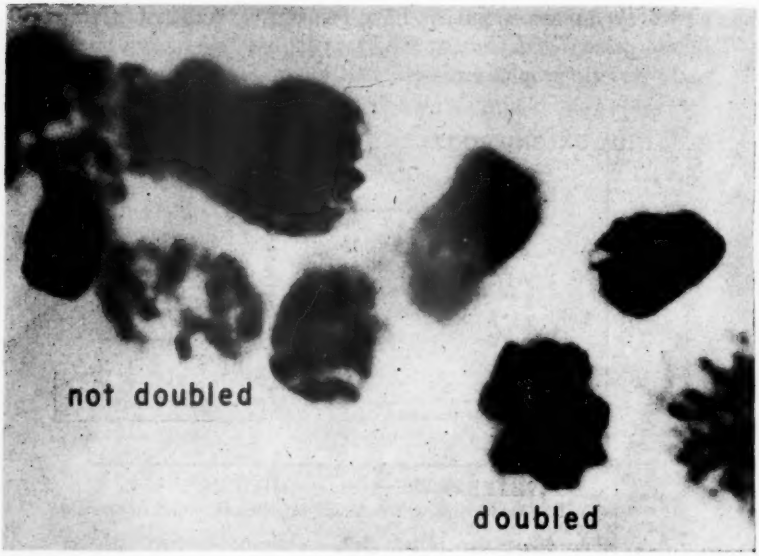

FIG. IX. Oogonial nuclei from the grasshopper *Melanoplus differentialis*, fixed in 10 per cent neutral formalin, and stained with the Feulgen reaction. The oogonial cell at left contains the diploid amount of DNA, and the cell at right twice the diploid amount. (From Swift and Kleinfeld, 1953.)

Distribution of DNA values in regenerating rat liver is strikingly similar to those found in some tumors, where polyploidy and mitosis may also occur together (Bader, 1953a). To give only one example, in the tumor shown in figure XI, Cloudman S-91 melanoma, both diploid and tetraploid metaphases may be seen. The DNA values (Fig. XII) show clear-cut classes, as liver, with the intermediate values between them probably representing cells in the process of DNA synthesis, and possibly also a small number of nuclei with chromosomal abnormalities. I would like to emphasize that in this tumor tissue the normal constant relation between chromosomes and DNA appears to be maintained. We cannot, therefore, blame

the extreme malignancy of the Cloudman melanoma on abnormal amounts of DNA in the component chromosomes.

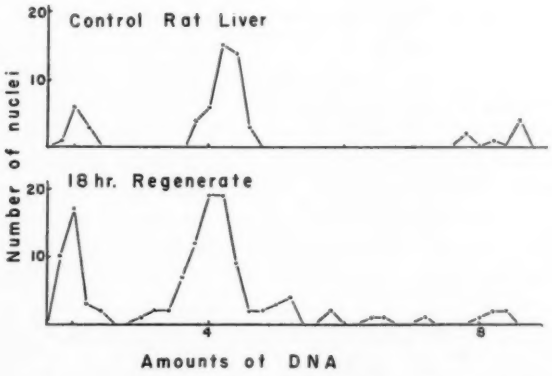

FIG. X. Amounts of DNA (Feulgen) in nuclei of normal and regenerating rat liver. Top, nuclei at time of operation, when left and middle lobes were removed. Bottom, nuclei of remaining right lobe from the same animal 18 hours later. (Data from Kleinfeld, unpublished.)

We have naturally been interested in the effects of various mitotic inhibitors on the pattern of DNA synthesis. In figure XIII a few representative values are plotted showing the effects of X rays and nitrogen mustard on DNA values in onion roots. An X-ray dose of 1,200 r was given actively growing root tips, causing a marked lowering in mitotic index within two hours, and a cessation of visible mitotic activity by 12 hours. Control DNA values show the expected spread, from the diploid level, through the zone of doubling, to twice the diploid amount, preparatory to mitosis. From 12 to 36 hours after X-ray treatment, however, all intermediate DNA values disappeared, although, as can be seen from the data given, the volumes remained approximately normal.

Intermediate amounts of DNA also disappeared when roots were treated with 2.6×10^{-4} M nitrogen mustard [bis (n-chloroethyl) methylamine hydrochloride] for 30 minutes, although in this case nuclear volumes, and also the total nuclear protein, continued to increase until abnormally large nuclei

were obtained. We may expect such agents as X-rays and nitrogen mustard to alter cells in many complex ways. These data, however, suggest that there are at least two sensitive times in the total mitotic cycle: one just preceding DNA synthesis, and another just preceding or in the early stages of mitosis. It seems probable that cells which had already commenced the process of DNA synthesis have completed the doubling in spite of the treatment (though they may also have regressed), just as we know that nuclei caught in the process of division during treatment, usually continue on into the subsequent resting phase. If this assumption is correct, we might also conclude that this process of duplication is commenced in all chromosomes of a nucleus at very nearly the same time, since the treat-

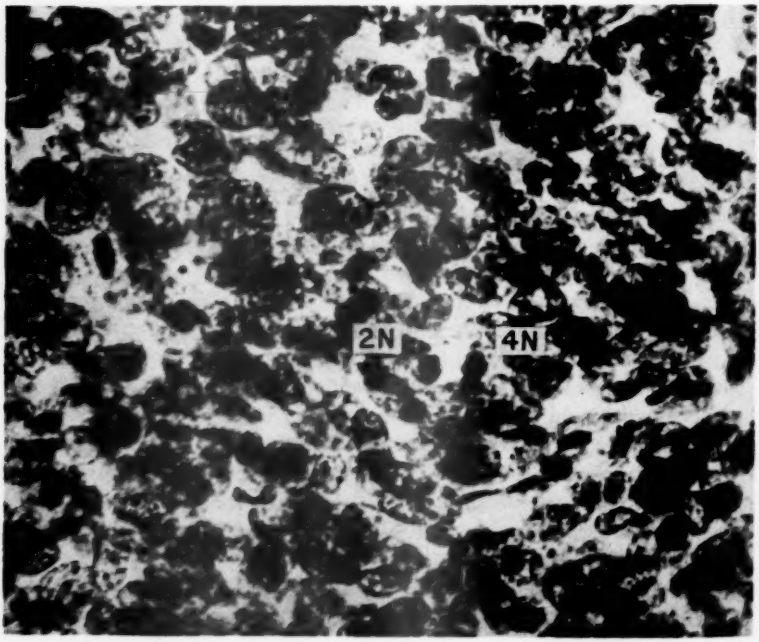

FIG. XI. Cloudman S-91 melanoma from a dba strain mouse, fixed in 10 per cent neutral formalin and stained with the Feulgen reaction. Metaphase figures with roughly the 2N and 4N number of chromosomes are visible.

ment seems sharply to divide the synthesized from non-synthesized cells.

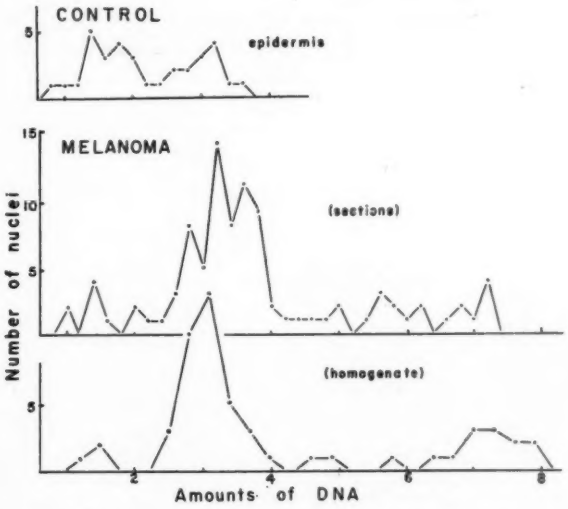

FIG. XII. Amounts of DNA (Feulgen) in nuclei of Cloudman S-91 melanoma, as compared with epidermis of the same animal. Tumor nuclei were measured both from sectioned material, and material homogenized in 30 per cent sucrose to reduce the irregular distribution of chromatin. (From Bader, 1953b.)

To summarize what I have said about the DNA fraction—in a wide variety of plant and animal nuclei there appears to be a constant relation between DNA and the chromosomes. We can say that a characteristic amount of DNA is associated with the chromosomes of each organism. The amounts of DNA are doubled prior to mitosis, and halved in sex cell formation. Where we have polyploid cells present within a tissue, the DNA amounts again parallel the chromosome numbers. I think we have studied enough nuclei to say that this quantitative behavior of DNA represents a general biological phenomenon. I certainly do not want to infer, however, that this constancy is rigidly obeyed by every living nucleus. There is certainly a tremendous amount we do not know about DNA,

and any exceptions to the constancy hypothesis will doubtless be of considerable interest.

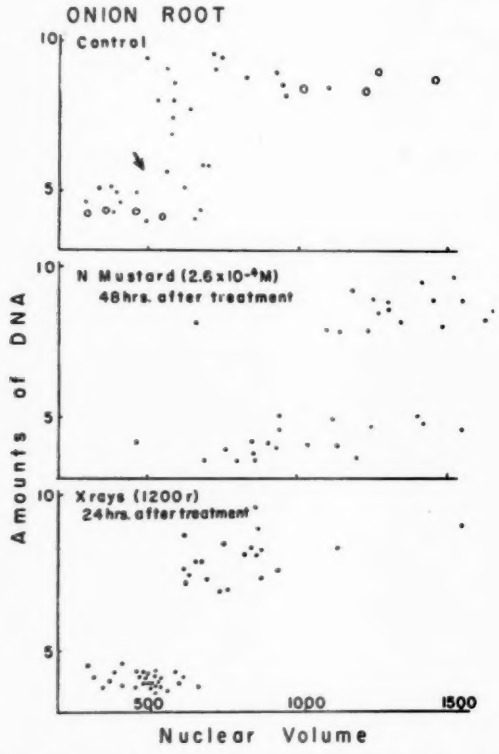

FIG. XIII. The relation between amounts of DNA (Feulgen) and nuclear volume in cubic microns in normal, nitrogen mustard- and X-ray-treated onion root tip meristems. Treatment in both cases has obliterated DNA values intermediate between diploid and twice the diploid value. After nitrogen mustard nuclear volumes have increased. Circles in top graph represent telophases (left) and prophases (right). (Data from Swift and Rasch, unpublished.)

RNA. The ribose nucleic acid occurs in at least three morphologically distinct parts of the cell: the cytoplasm, the nucleoli, and the chromosomes. It has been shown by many workers, notably Caspersson (1950) and Brachet (1947), that in

sharp contrast to the DNA, the amounts of RNA in the cytoplasm change markedly with altered cell states, being particularly high when cells are active in biosynthesis. Nucleolar RNA, as has been shown by many workers, follows the same pattern. Nucleoli are almost always large in rapidly growing tissues—for example, in embryos or tumors. In a few cases an increase in nucleolar RNA can be seen to precede an increase in cytoplasmic RNA. One such case is shown in figure XIV,

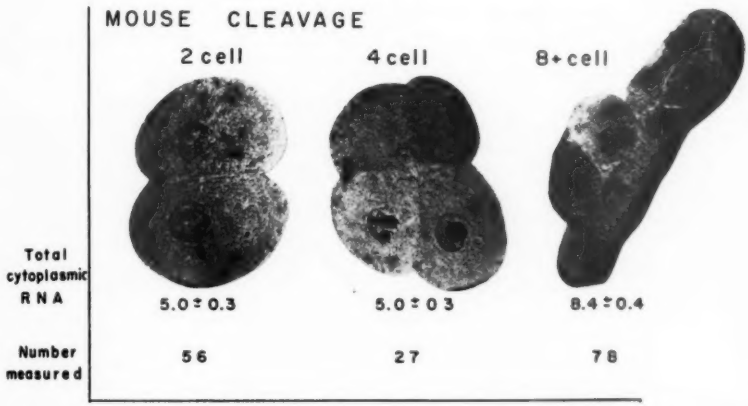

FIG. XIV. Mouse eggs in early cleavage, fixed in acetic-alcohol and stained with Azure B at pH 4.0. Nucleoli show marked increase in amount of dye bound between the 2 and 4 cell stage, but a significant increase in total cytoplasmic dye bound, associated with RNA, does not occur until about the 8 cell stage. (From Flax, 1953.)

in the cleavage stages of mouse embryos. During the 24 or so hours that elapse between the 2 and 4 cell stages, a marked change in the nucleoli is evident, although during this time the total RNA of the egg cytoplasm, as determined by Azure B, remains constant. It is not until about 24 hours later, at approximately the 8 cell stage, that a significant increase in total cytoplasmic RNA occurs. These data would support the concept, originally brought forward by Caspersson and associates, that cytoplasmic RNA is somehow mediated by the nucleolus.

Our measurements on the RNA fraction of the chromosomes or of interphase chromatin, excluding the nucleolus, are as yet preliminary. Table 1 shows RNA/DNA ratios from lily nuclei,

as determined by ultraviolet absorption, before and after enzymatic extraction. From these data two things are suggested. First, that unlike DNA, there appear to be differences in the amounts of RNA in different tissues, although measurements are not extensive enough as yet for statistical treatment. Second, during prophase of the ovary wall tissue there is not the marked increase in chromosomal RNA suggested by several other workers (*e.g.*, Jacobson and Webb, 1952). This chromosomal RNA fraction may prove of interest, then, as an indication of chromosomal activity—standing between the stability of the DNA fraction, and the highly variable RNA of nucleoli and cytoplasm.

TABLE 1

*DNA/RNA Ratios in Nuclei of Lilium henryi**

Tissue		Ratio	Mean
Ovary wall	Interphase	2.4	2.6
		2.5	
		2.6	
		2.8	
	prophase	1.4	2.3
		2.0	
		2.4	
		3.3	
Tapetum	interphase	2.0	3.5
		2.5	
		3.0	
		3.3	
		3.9	
		4.1	
		4.3	

*Measurements were made in ultraviolet light before and after extraction with desoxyribonuclease and ribonuclease. Absorption curves of the DNA and RNA extracted resembled those of nucleic acid, although a possible source of error due to light scatter plus anomalous dispersion has not been eliminated. (Data from Rasch and Swift, unpublished).

Although the nucleolar and cytoplasmic RNA fractions show marked fluctuations in total amount, closely related to fluctuations in synthetic activity of the cell, we know almost nothing of how RNA and protein synthesis are related on a biochemical level. A great deal more careful work remains to be done before these changes, which are now only suggestive, can be fitted into any sound interrelated system.

Proteins. Interphase nuclei may change in volume with changes of cell states, and nuclei of different tissues often have

a characteristic size, as shown by Jacobj many years ago. It is not surprising that measurements on the total protein of nuclei tend to parallel the nuclear volume as indicated by Schrader and Leuchtenberger (1950), and Alfert and Bern (1951). This is also shown for onion root nuclei in figure XV. Here

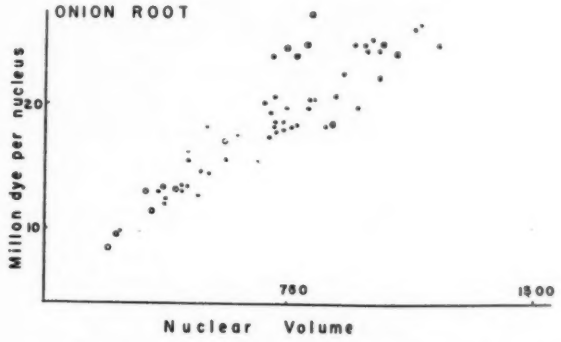

FIG. XV. The relation between Millon dye per nucleus and nuclear volume in cubic microns in cells from onion root meristem. Measurements were made at 500 mμ. Circles to left (below 750) telophases; to right, prophases.

total protein has been estimated by the Millon reaction which, we feel, is subject to several variables and thus can provide only a rough estimate. It seems, however, the best technique that we have found at present for measuring the protein of nuclei. We cannot distinguish chromosomal proteins and nuclear sap protein with this technique. One major chromosomal protein, the histone, contains only small amounts of tyrosine (Hamer, 1951), and so should not greatly influence the Millon intensity.

The Million values graphed in figure XVI indicate that two tissues we have found to contain similar amounts of DNA, the diploid cells of root cap and pericycle, vary quite markedly in their protein contents. Our measurements on division stages show essentially the pattern followed by DNA, with one exception—the values spread much more widely, subject to their position in the root tip. The same division into two equal daughter nuclei occurs, each with roughly half the protein of the prophase nucleus. In the *Tradescantia* root, nuclear volumes

of telophase nuclei are only about one-third of the prophase nucleus, so that some material appears to have been present in prophase but omitted from telophase nuclei.

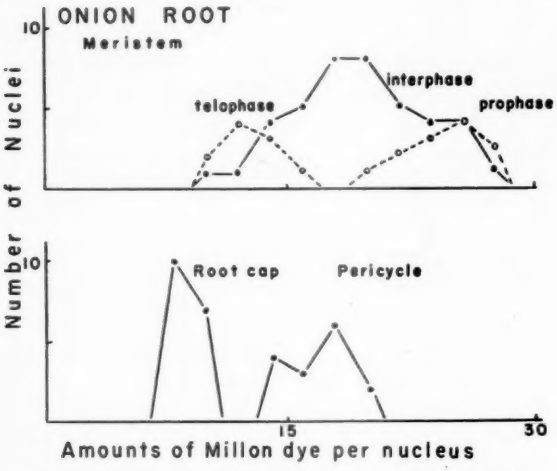

FIG. XVI. Amounts of Millon dye per nucleus in meristem and two differentiated tissues from onion roots.

In summary, I have tried to indicate some of the problems that can be studied by the comparatively recent techniques of cytophotometry. Our picture of cell nucleoproteins is still very fragmentary. Speaking in general terms, however, we can say that, while certain components fluctuate widely with altered cell states, certain others do not. Certain components, such as the total protein, seem to be duplicated only roughly in the mitotic cycle—in some cases duplicated in part before and in part after division. Other components, particular the DNA, seem to be doubled in a very special manner, in one specific period of interphase and preceding cell division. We have suggested that the compounds showing stability are associated with the stable genetic mechanism of the organism, and possibly are part of the genes themselves. The variable components, on the other hand, are certainly linked to the functional aspects of cells. For a credible picture of cell function we need to know

the mechanisms whereby the stable genotype is able to direct and control the numerous and complex cellular activities. We hope that studies of this type may at least get us one step nearer to the answer.

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CONCENTRATION AND ORGANIC BINDING OF RADIOIODIDE BY THYROID TISSUE*

SEYMOUR H. WOLLMAN†

The following paper summarizes results of some studies made in our laboratory to determine factors controlling the accumulation of radioiodine in normal and neoplastic thyroid tissue. Studies have been made mainly in mice[1] because a variety of transplantable tumors of the thyroid gland (Morris and Green, 1951) were available[2] in this species.

Studies have been made mainly of radioiodine uptake and of the ability of normal and neoplastic thyroid tissue to maintain a concentration of radioiodide elevated above that of the serum. The uptake of radioiodine is a measure of the ability of thyroid tissue to bind radioiodine in organic combination since almost all of the iodine or radioiodine in functional thyroid tissue is normally found in organic combination. The radioiodide concentration in the thyroid tissue appears of importance since the concentrated radioiodide is probably the I^{131} which becomes bound in organic combination. In addition, in situations in which the uptake of I^{131} is less than normal, as in many thyroid tumors, it is of interest to know whether the impairment is due to the lack of radioiodide in the tissue or to an impairment in the binding mechanism.

Radioiodide concentrating in thyroid glands of mice. The radioiodide in the thyroid gland of a mouse is maintained at a concentration many times the radioiodide concentration in the serum. This is observed whether organic binding is permitted or blocked by the injection of a single dose of propylthiouracil. The mechanism by which this concentration differ-

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† Present address: National Cancer Institute, Bethesda 14, Maryland.

[1] All experimental studies on mice have been made in collaboration with Dr. R. O. Scow of the National Institute of Arthritis and Metabolic Diseases.

[2] All tumor-bearing mice used in these studies were supplied by Dr. H. P. Morris of the National Cancer Institute.

ential is maintained is not known (Wollman and Scow, 1953b). However, it has been shown that in the mouse, radioiodide concentrating ability is only partially dependent upon the presence of the anterior pituitary in the host. Even after hypophysectomy the thyroid gland of the mouse can maintain a concentration of thyroid radioiodide over 50 times the radioiodide concentration in the serum (Wollman and Scow, 1953a).

Radioiodine uptake by transplantable thyroid tumors. Several lines of transplantable tumors of the thyroid gland were studied. Tumors of each line are histologically relatively uniform and free from necrosis. The tumor lines can be divided into two types on the basis of their requirements for growth:

(a) Dependent tumors will grow only if hosts are fed thiouracil.

(b) Independent tumors will grow in hosts fed a stock diet to which no goitrogen is added.

It was found by Wollman, Scow, Wagner, and Morris (1953) that the radioiodine uptake in tumors of a given line was proportional to the tumor weight provided that the uptake by the tumor was not too high. The uptake per mg. of tumor was a constant fraction of the uptake of the thyroid gland in the host independent of the time after I^{131} injection and of the tumor weight for the more active tumor lines. A non-functional tumor line was also studied. After I^{131} injection, the I^{131} content per mg. of tumors of this line decreased progressively during the period of observation; the tumor I^{131} content paralleled that of the blood rather than the thyroid. These results can be understood on the basis of a simple model in which the tumor clearance of radioiodide from the blood is proportional to the tumor mass with a constant of proportionality characteristic of the tumor line (Wollman, 1953a). The results of the experiments and the analysis appear to have important implications for radioiodine therapy of functional metastatic thyroid carcinomas in man (Wollman, 1953b).

Spot checks of radioiodine uptake in a variety of tumor lines indicated that all thyroid tumors had I^{131} uptakes per gram which were less than for the corresponding thyroids. Only tumors of dependent lines had uptakes per gram higher than 10 per cent of the uptake per gram of thyroid. The uptakes per

gram of independent tumors were less than those of the dependent tumors by another factor of 10 or more.

Radioiodide concentrating ability of transplantable thyroid tumors. Measurements of the ability of several lines of transplantable thyroid tumors to maintain a concentration of radioiodide elevated above the radioiodide concentration of the serum were made both when organic binding was permitted or when blocked by a single dose of propylthiouracil (Wollman, Scow, and Morris, 1953). It was found that tumors of a dependent line possessed considerable radioiodide concentrating ability, but that all independent tumors had lost the ability to concentrate radioiodide almost completely or completely, depending upon the tumor line. The data for tumors of a line undergoing transition from dependent to independent suggest that a major loss of radioiodide concentrating ability accompanies the transition to independence. The data are also in agreement with the hypothesis that in some independent tumors which can bind I^{131} the decreased binding of I^{131} is largely due to the low concentration of radioiodide present in the tumor tissue.

If the low radioiodide concentrating ability is, in fact, largely responsible for the decreased I^{131} uptake by independent thyroid tumors, it is important to determine how the ability can be increased. An attempt was made to induce radioiodide concentrating ability in tumors of a line which had lost the ability to concentrate iodide. Propylthiouracil feeding did not induce in the tumors the ability to concentrate radioiodide although it did increase the radioiodide concentrating ability of the thyroids in the same mice.

Radioiodine metabolism in the chick embryo. In the preceding section it was pointed out that when thyroid tumors become independent they undergo a marked loss of radioiodide concentrating ability, although they may retain the ability to incorporate I^{131} into organic combination. Comparison of properties of tumor and embryonic tissue have been routine in studies of the biochemistry of tumors. It therefore was of interest to see how closely embryonic thyroid compared with independent thyroid tumors in the ability to perform these two functions of thyroid tissue. The radioiodine metabolism of the chick embryo thyroid was examined at several developmental stages. It was

found that the thyroid gland of the 9-day embryo was able both to concentrate radioiodide and to incorporate it into organic combination (Wollman and Zwilling, 1953). On the other hand, the thyroid gland of the 7-day embryo was able to concentrate radioiodide but was not able to incorporate it into organic combination.

The 7-day chick embryo thyroid therefore appears to differ from independent mouse thyroid tumors in that in the embryonic thyroid the ability to concentrate radioiodide is well developed although the ability to bind is lacking, whereas, in the independent tumors, the ability to concentrate radioiodide is almost completely or completely lacking although the binding mechanism may be well developed.

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Items of Possible Interest to Our Friends

June, 1953

More or Less Basic: G Barac shows no matter how severe a jaundice, plasma contains enough albumin to bind all bilirubin in circulation (*Arch Internat Physio* 61/129/53). . . . K Burton & Co give free energy data for dehydrogenase systems (*Biochem J* 54/86, 94/53). . . . JH Cort finds serve stimulation reduces arterio-venus O_2 & CO_2 differences across kidney (*Nature* 171/784/May 2/53). . . . RE Coupland finds noradrenalin in para-aortic bodies (*J Endocrin* 9/194/53). . . . W Daubenmerkl notes spreading action of dihydroxymaleic acid (*Acta Pharmacol Tox* 9/1/53). . . . H Druckrey & Co analyse biochemorphology of cell-division inhibitors (*Arzneimittelf* 4/151/53). . . . KP DuBois & Co describe amidophyrophosphates of possible value in myasthenia gravis (*J Pharmacol* 107/464/53). . . . JD Ebert reports synthesis & distribution of species specific myosin in cardiac development (*Proc Nat Acad Sci* 39/333/53). . . . F Fiala & K Roth describe relation of platelets to thromboplastic system of coagulation (*Arch Internat Physical* 61/205/53). . . . M Gordon edits *Pigment Cell Growth* (Acad Press, NY, 53, 365pp, $9). . . . CM Grubers & KS Lee find papaverine coronary dilation & resp stimulation antagonize cardiac arrythmias of thiobarbital (*Arch Internat Pharmaco* 93/248/53). . . . BK Harned & Co (Lederle) report safe anticonvulsant action of benzyl chloropropionamide "Hibicon" (*J Pharmacol* 107/403/53). . . . E Jacobs & M Verbanck find parathyroid causes increase in rate of phosphate excretion (*Acta Med Scand* 145/143/53). . . . WJ Johnson & JH Quastel discuss narcotics & biological acetylations (*Nature* 171/602 Apr 4/53). . . . HA Krebs & Co describe phosphorylation quotient (*Biochem J* 54/107/53). . . . RA Miller shows relation of mitochondria to secretory activity in adrenal (*Am J Anat* 92/329/53). . . . JL Mongar & RF Whelon find d-tubocurarine increases plasma histamine while adrenalin does not (*J Physiol* 120/146/53). . . . MR Murray edits *Bibliography of Research in Tissue Culture* (Acad Press, NY, Sept 53, 2 vols, 2,000pp, $20). . . .N Onishi suggests arachnoid membrane & pia mater formed from connective tissue (*Fol Psych Neurol Jap* 5/263/53). . . . DM Prescott & A Zeuther find filtration constant higher than diffusion constant across egg membranes (*Acta Physiol Scand* 28/77/53). . . . JW Rebuck shows red cell structural changes on sensitization to specific antigens (*Anat Rec* 115/591/53). . . . Our EE Rennels finds phospholipids in anterior pituitary in acidiophilic secretory granules & Mitochondria (*ibid* p 659). . . . MRJ Salton notes more lipid in cell wall of gram-positive than in g-negative organisms (*Biochim Biophys* Acta 10/512/53).

More or Less Psychological: Bacterial warfare claims are presented (*Chinese Med J* 70/335–651/52, with 53 page supplement). . . . Sir Fredric Bartlett observes nature of thinking in medicine (*Brit Med J* 1/795/April/53), . . . Existentialist S de Beauvoir analyses *Marquis de Sade* (Grove

Press, NY, 53, 320pp, $5). . . . GS Blum considers *Psychoanalytic Theories of Personality* (McGraw-Hill, NY, 53, 219pp, $3.75). . . . L Berkowitz examines leadership sharing in small decision-making groups (*J Abn Soc-Psych* 48/231/53). . . . KM Colby salutes the Queen, in chess (*Psychoanal Rev* 40/44/53). . . . M Critchley discusses tactile thought with reference to blindness (*Brain* 76/19/53). . . . E Fagerberg suggests conditional reflex factors in psychology of bronchial asthma (*Acta Allerg* 6/61/53). . . . JD French & Co offer neural basis for anesthesia in block susceptible multi-synapic system (*AMA Arch Neurol Psych* 69/519/53). . . . RL Kirk & NS Stenhouse analyse genetic factors in ability to smell KGN (*Nature* 171/698/Apr 18/53). . . . E Podolsky edits *Encyclopedia of Aberrations: A Psychiatric Handbook* (Philosoph Libr NY, 53, 600pp, $10). . . . L Segy analyses emotional force of African sulpture in terms of latent animism (*Psyche* 8/108/53). . . . KW Schaie & Co describe differential mental decline in old age (*J Gerontol* 8/191/53). . . . JR Smythies discusses the experience & description of the human body (*Brain* 76/132/53). . . . KW Spence & JA Taylor confirm high manifest anxiety increases level of conditioning performance (*J Exp Psych* 45/265/53). . . . GR Stone develops theory of negative incentive in serial learning against punishment (*J Gen Psychol* 48/133/53). . . . J Wishner indicates correlation between Rorschach estimates & measures of physiological activity, suggesting psychopathology characterized by preponderance of diffuse unfocused activity (*J Abn Soc Psychol* 48/253/53).

More or Less Clinical: I Aird & Co show frequency of stomach cancer high in blood group A and low in O (*Brit Med J* 1/799/Apr 11/53). . . . HT Bloen & H Newman suggest choline in starvation or nephrotic edema (*Lancet* 1/827/Apr 25/53). . . . R Fischer suggests smegma as factor in cervical cancer (*Obs Gyn Rev* 8/232/53). . . . HF Fraser & JA Grider discuss treatment of drug addiction (*Am J Med* 14/571/53). . . . WG Lennox analyses febrile conculsions (*Pedi* 11/341/53). . . . OJ Pollak claims oral sitosterol inhibits intestinal absorption of cholesterol thus reducing blood cholesterol levels (*Circula* 7/702/53). . . . E Rodenwaldt edits *World-Atlas of Epidemic Diseases* (Falk-Verlag, Hamburg 1, 2 pts, 225DM, 53). . . . HA Weijars & Co study coeliac disease & find wheat factor harmful (*Acta Paed* 42/24, 34/53).

July, 1953

General: AE Berriman analyzes history of weights & measures in *Historical Metrology* (London, JM Dent, 53, 240pp, 16s). . . . RL Clark Jr & RW Cumley edit *The Book of Health*, a medical encyclopedia for everyone, well illustrated (Elsevier Press, Houston, 53, 850 pp, $10). . . . Our GH Crosby gives reactions to Jap internement camp life (*Colby Scholar* 1/16/53). . . . TF Currier & EM Tilton offer *A Bibliography of Oliver Wendell Holmes* (NY Univ Press, 53, 722pp, $20). . . . B Ghiselin anthologizes on *The Creative Process* (Univ California Press, Berkeley, 53, 322pp, $6.5). . . .W Goyen describes *The Enchanted Nurse* (*Southwest Rev* 38/

185/53). . . . SC Harvey & colleagues eulogize George H Smith (1885–1952), microbiologist (*Yale J Biol Med* 25/299–312/53). . . . W Haymaker edits *Neurological Problems in the World in 1953*, 59 articles by 75 authors, honoring Robert Wartenberg of San Francisco on his 65th birthday (*J Nerv Ment Dis* 116/469–1115/52). . . . PS Henshaw discusses physiologic control of fertility (*Science* 117/572/May 29/53). . . . A Koyre shows how successful experiments depend on sound theory (*Proc AM Philosoph Soc* 97/222/53). . . . E Larrabee discusses science, poetry & politics (*Science* 117/395/Apr 17/53). . . . Note Symposium on The Dean & the Psychiatrist (*Ment Hyg* 37/177–218/53). . . . I Thorner discusses ascetic protestantism & alcoholism (*Psychiatry* 16/167/53). . . . JH Woodger tackles *Biology & Language* (Cambridge Univ Press, 53, 422pp, $8).

CLINICAL: JF Ackroyd describes allergic purpura (*Am J Med* 14/605/53). . . . S Amano suggests development of annular cirrhosis from infectious hepatitis (*Acta Schol Med Univ Kioto* (30/183/52). . . . EJ Delorme shows fluctuations in red cell content of tissues in bleeding & transfusion & decrease in muscles on stimulation of motor cortex (*Quart J Exp Physiol* 38/47/53). . . . V Erspamer & A Ottolenghi find hydroxytrytamine specific hormone of entero-chrommaffin cells controls intrarenal blood flow & acts as antidiuretic (*Arch Internat Pharmaco* 93/177/53). . . . E Gellhorn offers *Physiological Foundations of Neurology & Psychiatry* (Univ Minnesota Press, Minneapolis, 53, 556pp, $8.5). . . . MF Hickey discusses genes & mermaids: theories of congenital abnormalities (*Med J Austral* 1/649/May 9/53). . . . HB Peck describes group therapy in clinic service (*Am J Orthopsych* 23/338/53). . . . AB Sabin introduces symposium on toxoplasmosis (*Am J Trop Med Hyg* 2/360–444/53). . . . M Stefanini & W Dameshek report on platelet transfusion, recommending direct transfusion of polycythemic blood with multiple silicone coated syringes (*New Eng J. Med* 248/797/May 7/53). . . . H Thaler describes histology of virus hepatitis (*Schw Ztschr allg Path Bakt* 16/129/53).

EXPERIMENTAL: ZM Bacq & Co note mercapto-ethylamine protects against X rays & helps in radiation sickness (*Science* 117/633/June 5/53). . . . F Barron shows personality correlates of judgment independence; yielders being practical, physicalistic, group oriented, while independents are creative, artistic, individualistic (*J Perosnal* 21/287/53). . . . L von Bartalanffy & RR Estwick relate tissue respirations of muscles to body size (*Am J Physiol* (jazzed-up) 173/58/53). . . . P Bergman shows spermigration optimal in water phase of cervical mucouc during thermal shift at ovulation (*Fert Ster* 4/183/53). . . . EW Campbell & M Stefanini isolate plasma thromboplastin (*Proc Soc Exp Biol Med* 83/105/53). . . . Our CE & O Hall describe parabiotic hypertension after total adrenalectomy (*Am J Physiol* 173/29/53). . . . ML Hesselbach & HG duBuy localize glycolytic & respiratory enzyme systems on isolated brain mitochondria (*Proc Soc Exp Biol Med* 83/62/53). . . . HE Himwich discusses behavior effects of di-isopropyl fluorophosphate & atropine (*Arzneimittelforsch* 3/228/53. . . . DJ Morton & DD Fuller discuss *Human Locomotion & Body Form: A Study*

of Gravity & Man (Wms & Wilkins, Balt, 53, 295pp, $5). . . . Our KP McConnell & Co, using selenium fixation by red cells, estimate intravascular life span at 11 days (*Proc Soc Exp Biol Med* 83/140/53). . . . HR Nevanlima discusses factors affecting maternal Rh immunization (*Ann Med Exp Biol Fenn,* 31, Suppl 2, Helsinki, 53, 80pp). . . . CA Nichol finds liver converts pteroylglutamate to citrovorum factor (*Proc Soc Exp Biol Med* 83/167/53). . . . JR Robinson analyses active transport of water in living systems (*Biol Rev* 28/158/53). . . . A Szent-Gyorgyi offers *Chemical Physiology of Contraction in Body & Heart Muscle* (Academic Press, NY, 53, 159pp, $4.8). . . . JL Tullis edits *Blood Cells & Plasma Proteins: Their State in Nature* (Academic Press, NY, 53, 438pp, $10). . . . EJ Van Liere & Co find semistarvation increases propulsive motility of small intestine (*Proc Soc Exp Biol Med* 83/98/53). . . . JM Yoffey edits symposium on *The Suprarenal Cortex* (Academic Press NY, 53, 240pp, $6.8).

AUGUST, 1953

GENERAL: EM Bell reviews rise of women medicos in *Storming the Citadel* (Constable, London, 53, 200pp, 18s). . . . EF Brown & Co ask if midwives should be ignored, eliminated or controlled (*South Med J* 46/639/53). . . . N Bull develops attitude theory of emotion (Monog 81, *Nerv & Ment Dis Monog,* NY, 53, 312pp, $6). . . . HR Cox reviews viral vaccines in human welfare (*Lancet* 265/1/July 4/53). . . . HJ Eyseneck notes difficulties in *The Scientific Study of Personality* (Routledge, London, 53, 333pp, 30s). . . . TR Forbes discusses social history of the caul (*Yale J Biol Med* 25/405/53). . . . SC Harvey & CW Barker offer tribute to Herbert Thoms (*ibid* pp447–467). . . . JW Higgins suggests alcoholism is psychic defense against sources of anxiety (*AMA Arch Neurol Psychi* 69/713/53). . . . M Jones discusses *Social Psychiatry* (Tavistock Publ., London, 53, 183pp, 20s). . . . AC Kinsey & Large Co announce *Sexual Behavior in the Human Female* (meaning white women in eastern US) (WB Saunders, Phila, 53, 846pp, $8). . . . K Kleist resolves DuBois Reymond's difficulty on brain & psyche (*J Nerv Ment Dis* Wartemberg Festschrift, 116/776/53). . . . MT Moore discusses much on epilepsy (*ibid* p 841). . . . W Penfield gives educational consequences of neurophysiological mechanisms of speech (*Proc Am Acad Arts Sci* 85/199/53). . . .N Rashevsky outlines mathematical approach to history (*Bull Math Biophysics* 15/197/53). . . . KE Rothschuh surveys *Geschichte de Psysiologie* (Springer, Berlin, 53, 260pp, DM 29.6). . . . M Seeman raises question of role conflict & ambivalence in leadership (*Am Soc Rev* 18/373/53). . . . Note Symposium on influence of George Berkeley (1684–1753) (*Brit J Philos Sci* 4/1–91/53). . . . AS Votos & J Glenn describe group technique in overcoming medical students' resistance to learning psychiatry (*Internat J Group Psych* 3/293/53). . . . P Wallin & HM Vollmer find greater attachment for mother than for father as parental happiness declines (*Am Soc Rev* 18/424/53). . . . Interesting to all is WA Westley's analysis of violence & the police (*Am J Sociol* 59/34–41/53). . . . Wellcome Foundation announces centenary exhibit

honoring Sir Henry Wellcome (1853–1936) at Wellcome Research Institute, 183 Euston Rd, London, & sp exhibits at Wellcome History of Medicine Museum, 28 Portman Sq, London.

CLINICAL: WH Abelmann & Co develop symposium on mitral valve malfunction (*Am J Med* 15/5–76/53). . . . JP Barker EF Adoph find seawater aids survival on sole diet of dried milk (*Am J Physiol* 173/495/53). . . . L Dexter praises surgery in handling mitral stenosis (*Arch Inst Cardiol Mex* 23/193/53). . . . R Fischer & Co note hair loss in anticoagulant therapy (*Schw Med Wchnschr* 83/509/May 30/53). . . . RW Gerard & RE Taylor survey recent progress in nerve-muscle physiology (*Am J Med* 15/83/53). . . . HM Gerson has evidence of successful management of peptic ulcer with desiccated pineal & tuber cinereum (*Acta Med Scand* 145/370/53). . . . J Kinning & H Rieth review antimycotics (*Arzneimittelforsch* 3/267/53). . . . RW Luxton describes radiation nephritis (*Quart J Med* ns22/215/53). . . . AIG McLaughlin discusses prevention of dust diseases (*Lancet* 2/49/July 11/53). . . . CS Nadler reviews potassium metabolism (*Am J Med Sci* 266/88/53). . . . RM Nelson & D Seligson study blood ammonia in normal & shock states (*Surg* 34/1/53). . . . AJ Plummer & Co report on useful safe anticholinergic & antiulcer action of "Antrenyl" a methylammonium bromide (*J Pharmacol* 108/292/53). . . . H Staub surveys chemotherapy of TB (*Schw Med Wchnschr* 83/531/June 15/53). . . . IMG Stewart discusses headache in hypertension (*Lancet* 1/1261/June 27/53). . . . HS Sullivan's (1892–1949) *Interpersonal Theory of Psychiatry* is edited by HS Perry & ML Garvel (WW Norton, NY, 53, 393pp, $5). . . . R Tagnon discusses adrenal cortex effects in diabetes in resensitivity to insulin (*Acta Clin Belg* 8/105/53). . . . R Wegria & Co suggest electric countershock & massage best for cardiac resuscitation in ventricular fibrillation (*Circul* 8/1/53). . . . G Westmark appraises heredity, physique & personality of peptic ulcer individuals (*Acta Psych Neurol Scand*, Suppl 84, Copenhagen, 53, 183pp).

SEPTEMBER, 1953

CLINICAL AND PRECLINICAL: LG Abood & RW Gerard find diphenyl cmpds as benadryl are strong cytochrome oxidase inhibitors (*J Pharmacol* 108/261/53). . . .H Baxter & Co prefer exposure to occlusive dressing in treating burns (*Canad Med Asso J* 69/97/53). . . . K Billerbeck reviews morphological changes in mesenchyme in immunobiological defense (*Artzforsch* 7/303/53). . . . KF Bonhoeffer offers model of nerve excitation (*Naturwiss* 40/301/53). . . . R Bowen shows individuality in allergy in identical twins (*J Allerg* 24/236/53). . . . K Bradley & JC Eccles propose strychnine action as depression of primary inhibition (*Nature* 171/1061/June 13/53). . . . JH Burn says local acetylcholine regulates heart rhythm (*Lancet* 1/1161/June 13/53). . . . G Butler & Co sympose on nucleoproteins (*Canad J Med Sci* 31/222–305/53). . . . PN Campbell & TS Work review biosynthesis of proteins (*Nature* 171/997/June 6/53). . . . C Carruthers & V Suntzeff review Biochemistry & Physiology of Epidermis (*Physiol*

Rev 33/229/53). . . . LJ Cass & WS Frederik find dextromethorphan HBr 4 mgm orally 4 times daily is effective & safe antitussive (*New Eng J Med* 249/132/July 23/53). . . . SR Dasgrupta & Co analyse sympatholytic action of Rauwolfia Serpentina (*Ind J Med Sci* 7/220/53). . . . C deDuve reports on glucagon, hyperglycemic glycogenolytic factor of pancreas (*Lancet* 2/99/July 18/53). . . . FA Denz finds sp cholinesterase as main enzyme at myoneural junction (*Brit J Exp Path* (34/329/53). . . . E DeRobertis & Co show thrombin lyses platelets (*Blood* 8/587/53). . . . B Ephrussi describes *Nucleo-cytoplasmic Relations in Micro-organisms: Their Bearing on Cell Heredity & Differentiation* (Oxford Univ Press, London, 53, 210pp, 18s). . . . JA Frank omits references (*Calif West Med* 31/27/29; *Anesth Analg* 9/62/30) in review of CO_2 therapy in mental disorder (*Am J Psych* 110/93/53). . . . Our Funan Hu & Co show comparative toxic effects of antibiotics on tissue cultures of human skin (*J Invest Dermat* 20/357/53). . . . J Furth reviews conditioned & autonomous neoplasms (*Cancer Res* 13/477/53). . . . WH Gaylord Jr & JL Melnick describe intracellular pox viruses (*J Exp Med* 98/157/53). . . . MA Gerebtzoff & J LeMagnen review structure of olfactory organ & psycho-physiological regulation of mechanism of olfaction (*J Physiologie* 45/247/53). . . . E Grastyan & Co show motor inhibitory center in ventral hypothalamus (*Acta Physiol Acad Sci Hung.* 4/241/53). . . . O Hechter edits symposium on mechanism of cortico-steroid action in disease (*Ann NY Acad Sci* 56/623–814/53). . . . SW Hoobler & AS Dontas survey drug treatment of hypertension (*Pharmacol Rev* 5/135/53). . . . JAA Ketelaar discusses *Chemical Constitution* (Elsevier, Houston, 53, 400pp, $5.5). . . . G Loewi finds inverse relation between chondroitin in human cartilage & polymerization with age, suggesting depolymerization favors calcification (*J Path Bact* 65/381/53). . . . CA Montagnani & FA Simeone note mychemoglobin release after muscle ischemia with possible kidney injury (*Surg* 34/169/53). . . . FD Mann reviews clinical assay of blood coagulation factors (*Am J Clin Path* 23/623/53). . . . K Motokawa & M Ebe analyse mechanism of apparent movement (*J Exp Psych* 45/378/53). . . . MR Murray & G Kopeck compile *A Bibliography of the Research in Tissue Culture* (Academic Press, NY, 53, 2 vols, 2000pp, $24). . . . Sir Rudolph Peters arranges symposium on enzyme research (*Brit Med Bull* 9/85–164/53). . . . A Pletscher says fructose hastens metabolism of alcohol via pyruvate (*Helv Med Acta* 20/100/53). . . . W Raab offers *Hormonal & Neurogenic Cardiovascular Disorders* (Wms & Wilkins, Balt, 53, 750pp, $15). . . . JF Riley & GB West show tissue mast cells rich in histamine (*J Physiol* 120/528/53). . . . WC Schneider & Co find alkaline phosphatase concentrated in Golgi substance (*Nature* 172/161/July 25/53). . . . W Seifriz discusses rhythmic impulses in protoplasm (*ibid* 171/1136/June 27/53). . . . T Sjostrand reviews blood volume in regulating circulation (*Physiol Rev* 33/202/53).

GENERAL: J Brozek discusses measurement of nutriture (*Am J Physical Anthropol* 11/147/53). . . . AJ Carlson says increasing understanding of man & nature adds to scientists' social responsibility (*Science* 177/701/June 26/ 53). . . . E Clark is delightful as *Lady with a Spear* (Harper, NY, 53,

253pp, $3). . . . AC Crombie shows scientific progress in *Augustine to Galileo* (Falcon, London, 53, 451pp, 42s). . . . M Culpin & M Smith describe *The Nervous Temperament* (Indust Health Res Bd Rep 61, London, 53, 80pp, 4s6d). . . . S Davis indicates antiquity of snake cult in Greece & near East (*Scientia* 88/83/53). . . . JE Deitrick & RC Berson survey *Medical Schools in the United States at Mid-Century* (McGraw-Hill, NY, 53, 390pp, $4.5). . . . I Galdston edits important *Epidemiology of Health* (NY Acad Med, NY, 53, 197pp, $4). . . . SK RamachandraRao describes psychological speculations of Samkara 788–820 AD (*Scientia* 88/141/53). . . . D Rapaport discusses psycho-analytic theory of emotion ,affects) (*Internat J Psycho-Anal* 34/177/53).

OCTOBER, 1953

INTERNATIONAL CONGRESSES: Proceedings of First World Conference on Medical Education (London, Aug 22–29) well reviewed in *Brit Med J* (Aug 29, Sept 5/53), to be published by Oxford Univ Press. At the XIXth International Congress of Physiology at Montreal, Sept. 1–6, there was distributed *Jacques Cartier et "La Grosse Maladie,"* containing a facsimile of *Brief recit* (Paris, 1545, 96pp), with JL Launay's English translation of the portion describing an outbreak of scurvy and its treatment by a decoction obtained from a tree identified by J Rousseau as Arbor vitae. At the Sixth International Congress of Microbiology at Rome, Sept 6–12, delegates received CF Cogressi's *Nuova Idea del Male Contagioso de' Buoi*, in facsimile, with an English translation by DM Schullian, & historical introduction by L Belloni.

GENERAL: EH Ackerknecht does right by *Rudolf Virchow: Doctor, Statesman, Anthropologist* (Univ Wisconsin Press, Madison, 53, 320pp, $5). . . . N Brennan observes *The Making of a Moron* (Sheed & Ward, NY, 53, 145pp, $2.5). . . . J Bronowski notes *The Common Sense of Science* (Harvard Univ Press, Cambridge, 53, 154pp, $2). . . . CIC de Courcy translates G Revesz's *Introduction to the Psychology of Music* (Longmans Green, London, 53, 334pp, 30s). . . . W Durant offers 5th Vol of *Story of Civilization, The Renaissance* (Simon & Schuster, NY, 53, 522pp, $7.5). . . . J Ek & B Josephson find beer increases sodium but decreases potassium excretion (*Acta Physiol Scand* 28/355/53. JR Foster edits *The World's Great Folktales* (Harper, NY, 53, 340pp, $3.75). . . . JF Fulton & Co describe AL Lavoisier's work on ether (*J Hist Med* 8/318/53). . . . E Gilson sings with *Choir of Muses* (Sheed & Ward, NY, 53, 219pp, $3.5). . . . D Mckie describes *Antoine Lavoisier: Scientist, Economist & Social Reformer* (Constable, London, 53, 320pp, 30s). . . . E Podolsky edits *Encyclopedia of Aberrations: A Psychiatric Handbook* (Philosophical Library, NY, 53, 645pp, $10). . . . CE Raven analyses *Experience & Interpretations* (Cambridge Univ Press, London, 53, 230pp, 21s). . . . R Roy exposes *Apostles of Discord* (Beacon Press, Boston, 53, 440pp, $3.75). . . . M Sarton's poetry pleases in *The Land of Silence* (Rinehart, NY, 53, 148pp, $2.5). . . . GE Swanson has evidence that participants acquaintance with task & with each other helps

smooth group behavior (*Am Social Rev* 18/522/53). . . . SA Waksman well reviews streptomycin (*Science* 118/259/Sept 4/53).

CLINICAL: AL Barach & Co devise exsufflation with negative pressure (EWNP) surpassing cough to expel bronchial secretions impairing respiration (*Am J Med Sci* 226/241/53). . . . JM Barnes describes ***Toxic Hazards of Certain Pesticides to Man*** (WHO Monogr Ser 16, Geneva, 53, 129pp with 689 references). . . . HK Beecher proposes method for quantifying intensity of pain (*Science* 118/322/Sept 18/53). . . . L Bellak edits ***Psychology of Physical Illness*** (Grune & Stratton, NY, 53, 256pp, $5.5). . . . WMC Brown & RF Mahler find radiation effects of X-ray dose depend on body size & suggest diffusible metabolite involved (*Science* 118/271/Sept 4/53). . . . E Endroczi & K Lissak find epileptic patients more sensitive than normals in pituitary-adrenal cortex response to stress (*Acta Physiol Acad Sci Hung*. 4/271/53). . . . JW Howard describes his new ink-blots for group testing (*J Clin Psychol* 9/209/53). . . . HE Johns analyses ***The Physics of Radiation Therapy*** (CC Thomas, Springfield, Ill., 53, 298pp, $6). . . . DG McKay & Co suggest pathologic anatomy of eclampsia with renal & pituitary necrosis is related to generalize Schwartzman phenomenon (*Am J Obs Gyn* 66/507/53). . . . H Miller & DW Baruch describe psychotherapy in acute bronchial asthma (*Ann Allergy* 11/438/53). . . . Pharmaceutical Press offers *Antibiotics: A Survey of Their Properties & Uses* (London, 53, 299pp, 26s). . . . T Poulson finds synergism between carbon dioxide & ether on reflex irritability (*Acta Pharmacol Toxicol* 9/148/53). . . . JF Riley notes tissue mast cells contain heparin & histamine (*Science* 118/332/Sept 18/53). . . . FE Sammis describes ***The Allergic Patient & His World*** (CC Thomas, Springfield, Ill., 53, 166pp, $3). . . . C Schroeder & H Heckel suggest EEG to diagnose birth trauma in new born (*Klin Wchnschr* 31/808/Sept 1/53). . . . JK Shen & Co recommend starch sponges as hemostatic agents (*Chinese Med J* 71/167/53). . . . MM Stern discusses trauma & symptoms formation (*Internat J Psycho* 34/202/53). . . . F Wengraf writes *Psychosomatic Approach to Gynecology & Obstetrics* (CC Thomas, Springfield, Ill., 53, 361pp, $7). . . . HA Wilmer says ***This Is Your World*** to the chronically ill (CC Thomas, Springfield, Ill., 53, 165pp, $3).

NOVEMBER, 1953

PRECLINICAL: P Adler & J Straub suggest non-fluorine water-borne caries-protective agent (*Acta Med Acad Sci Hung*. 4/221/53). . . . H Braunsteiner & Co show cytoplasmic filamentous structure in bone marrow & plasma cells (*Blood* 8/916/53). . . . H Croxatto & Co find pregnancy inactivates antidiuretic hormone (*Proc Soc Exp Biol Med* 83/184/53). . . . H Dale discusses a chemical phase in transmission of nervous effects (*Endeavor* 12/117/53). . . . B Ephrussi discusses *Nucleo-Cytoplasmic Relations in Microorganisms* (Oxford Univ Press, London, 53, 187pp, 18s). . . . EJ Kempf edits symposium on Comparative Conditioned Neuroses (*Ann NY Acad Sci* 56/141/380/53) & defines neuroses as conditioned conflicting holistic attitudinal acquisitive-avoidant reactions (*ibid* p 307). . . . A Klein &

R Arnheim undertake perceptual analysis of a Rorschach card (*J Personal* 22/60/53). . . . AGE Pearse gives *Histochemistry: Theoretical & Applied* (Little Brown, Boston, 53, 520pp, $12). . . . EC Pollard describes *The Physics of Viruses* (Acad Press, NY, 53, 200pp, $6). . . . Our M Pollard & RH Bussell find antineoplastic factor in spleens of mice treated with carcinogen (*Proc Soc Exp Biol Med* 83/671/53). . . . Our CM Pomerat notes rotation of cell nuclei (*Exp Cell Res* 5/191/53). . . . S Roberts & CM Szego review steroid interaction in metabolism of reproductive target organs (*Physiol Rev* 33/593/53). . . . E Stori & Co find normal megakaryocytes & platelets contain polysaccharide granulations (*Acta Haematol* 10/144/53). . . . A Wilson & Co show thymus extract depresses nerve muscle function (*Lancet* 2/368/Aug 22/53).

CLINICAL: J Brod indicates cerebral factors in regulation of renal function (*Acta Med Acad Sci Hung.* 4/369/53). . . .EB Brown surveys physiological effects of hyperventilation (*Physiol Rev* 33/445/53). . . . KA Fredrickson suggests iron tolerance test in difficult gastric diagnoses, being low in cancer (*Acta Med Scand* 146/259/53). . . . GBJ Glass & LJ Boyd treat pernicious anemia with low dose cobalamin with mucinous material frorm stomach (*Blood* 8/867/53). . . . S Hafez summarizes hormonal mechanisms of mammary development & lactation (*Acta Endocrine* 13/193/53). . . . GJ Hamwi & S Urbach recommend lean body mass & cell mass as physiologic reference factors for metabolic studies (*Metab Clin Exp* 2/391/53). . . . J Mayer surveys genetic, traumatic, & environmental factors in obesity (*Physiol Rev* 33/472/53). . . . V Menkin discusses accumulation of ACTH & cortisone in inflamed areas (*Brit J Exp Path* (34/420, 412/53). . . . EE Muirhead describes patterns of renal salt loss in renal failure (*Geriatrics* 8/471/53). . . . JLN Petisca notes localized eosinophilia (*Arq Patol* 24/432/53). . . . JP Soulier & MJ Larrieu differentiate 2 types of hemophilia on basis of missing profactor of thromboplastin (*New Eng J Med* 249/547/Oct 1/53). . . . A Wieder edits *Contributions toward Medical Psychology: Theory & Psychodiagnostic Methods* (Ronald Press, NY, 53, 2 vols, 907pp, $15).

GENERAL: GS Blum presents *Psychoanalytic Theories of Personality* (McGraw-Hill, NY, 53, 219pp, $3.75). . . . FJ Braceland reviews influence of hormones on emotions (*Bull NY Acad Med* 29/765/53). . . . FW Clement describes surgery before anesthesia (*Anesth* 14/473/53). . . . HH Dale's *Adventures in Physiology* contains fine introduction & bibliography with selection of contributions (Pergamon Press, London, 53, 678pp, $17). . . . HJ Eyseneck offers *The Scientific Study of Personality* (Routledge, Kegan Paul, London, 53, 320pp, 30s). . . . NN Foote suggests ambivalence in love is peculiar to our competitive society & may be made to disappear (*Psychiatry* 16/245/53). . . . T Heyerdahl domuments *American Indians in the Pacific: The Theory behind Kon-Tiki* (Rand-McNally, Chicago, 53, 821pp, $15). . . . R Jarrell analyses *Poetry & the Age* (Knopf, NY, 53, 271pp, $4). . . . ME Jones biographizes Albrecht Kossel (1853–1927) (*Yale J Biol Med* 26/80/53). . . . HC Kelman studies attitude change as a function of response restriction, finding learning favored by student thinking

& positive student-teacher relation (*Human Rel* 6/185/53. . . . LS Kubie calls for maturity in psychiatric research (*J Med Educ* 28/11/Oct 53). . . . JM Maclachlan edits *Health in the Later Years* (Univ Florida Press, Gainesville, 53, 123pp, $1.5). . . . J Ruesch reviews theory of human communication (*Psychiatry* 16/215/53). . . . H Strughold notes Martian atmosphere like ours ten miles up may support some life (*The Green & Red Planet*, Univ New Mexico Press, 53, 107pp, $4). . . . M Taffel eulogizes Yale surgeon, Samuel Clark Harvey, 1886–1953 (*Yale J Biol Med*, 26/1/53). . . . GK Tallmadge organizes exhibit on *Story of Medicine in Art* (Milwaukee Art Inst, Milw, Wisc, 53, 64pp, 25c). . . . RA Tsanoff comments on *The Great Philosophers* (Harpers, NY, 53, 653pp, $6). . . . CH Waddington describes the evolution of adaptations (*Endeavor* 12/134/53). . . . RI Watson gives account of clinical psychology in this century (*Psych Bull* 50/321/53).

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